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CONTENTS

NUMBER 1, SEPTEMBER, 1911

I. The Effects of Pilocarpine and Atropine upon the Amylolytic Power and the Composition of the Saliva. By E. M. Ewing.....	1
II. The Actions of Caffein on the Mammalian Circulation. I: The Persistent Effects of Caffein on the Circulation. By Torald Sollmann and J. D. Pilcher.....	19
III. A Consideration of Some Biologic Tests for Epinephrin. By. R. G. Hoskins.....	93

NUMBER 2, NOVEMBER, 1911

IV. Studies on the Pharmacological Action of Oxidizing Substances. By A. S. Loevenhart and W. E. Grove.....	101
V. The Action of Hydrocyanic Acid on the Respiration and the Antagonistic Action of Sodium Iodosobenzoate. By W. E. Grove and A. S. Loevenhart.....	131
VI. On the Antiseptic and Bactericidal Action of the Sodium Salts of Iodbenzoic, Iodosobenzoic and Iodoxybenzoic Acids. By Aaron Arkin.....	145
VII. A New Water Soluble Active Constituent of Squills. By Arthur James Ewins.....	155
VIII. The Physiological Action of Phenolphthalein Oxime. By M. Dresbach.....	161
IX. On the Convulsant Action of Acid Fuchsin upon Frogs Deprived of Their Cardiac Circulation. By D. R. Joseph and S. J. Meltzer.....	183

NUMBER 3, JANUARY, 1912

X. The Physiological Action of Cytisine, the Active Alkaloid of Laburnum (Cytisus-Laburnum). By H. H. Dale and P. P. Laidlaw.....	205
XI. The Influence of Sodium Iodoxybenzoate and Sodium Cyanide upon an Allergic Reaction of Inflammatory Character. By Samuel Amberg and J. H. Mason Knox, Jr.....	223
XII. Alcohol and Caffein: A Study of Antagonism and Synergism. By J. D. Pilcher. With a Discussion by Torald Sollmann.....	267
XIII. Physiological Studies in Anaphylaxis. IV. Reaction of the Cat toward Horse Serum. By. W. H. Schultz.....	299
XIV. Two Crystalline Pharmacological Agents Obtained from the Tropical Toad, Bufo Agua. By John J. Abel and David I. Macht.....	319

NUMBER 4, MARCH, 1912

- XV. A Note on the Effect of Nicotine Injection on Adrenal Secretion. By W. B. Cannon, J. C. Aub, and C. A. L. Binger..... 379
- XVI. Quantitative Studies on the Gastro-Intestinal Absorption of Drugs. II. The Absorption of Sodium Iodide. By Paul J. Hanzlik..... 387
- XVII. A Study of the Action of Various Diuretics in Uranium Nephritis. By William de B. MacNider..... 423
- XVIII. Isocalycanthine and Its Quaternary Base. By Hugh McGuigan and C. L. von Hess..... 441
- XIX. Scientific Proceedings of the American Society for Pharmacology and Experimental Therapeutics. Edited by the Secretary, Dr. John Auer. 455

NUMBER 5, MAY, 1912

- XX. The Pharmacological Action of Vanadium. By D. E. Jackson..... 477
- XXI. Studies on the Convulsive Reflex Produced by Strychnine. I. Habit. By H. T. Mostrom and H. McGuigan..... 515
- XXII. Studies on the Convulsive Reflex Produced by Strychnine. II. As Modified by Epinephrine. By H. T. Mostrom and H. McGuigan..... 521
- XXIII. On the Convulsant Action of Some Sulphonated Dyes. By David I. Macht..... 531
- XXIV. The Seat of the Emetic Action of Apomorphine. By Cary Eggleston and Robert A. Hatcher..... 551
- XXV. On the Action of Drugs and the Function of the Anterior Lymph Hearts in Cardiectomized frogs. By John J. Abel..... 581

NUMBER 6, JULY, 1912

- XXVI. The Action of Caffein on the Mammalian Heart. By J. D. Pilcher.. 609
- XXVII. The Action of Glandular Extracts upon the Amount of Epinephrine in the Blood. By Isaac Ott and John C. Scott..... 625
- XXVIII. On the Action of Sodium Citrate upon Mammalia, with Especial Reference to Acquired Tolerance and to Its Action upon the Cerebellum. By T. Brailsford Robertson and Theo. C. Burnett..... 635
- XXIX. The Action of Salts of Choline on Arterial Blood Pressure. By Lafayette B. Mendel, Frank P. Underhill and R. R. Renshaw..... 649

ILLUSTRATIONS

Effects of caffeine on respiration and blood pressure (Experiment C, 110-1) (Fig. 1).....	21
Effects of caffeine on spleen-volume (upper tracing), and mean blood pressure (Experiment C, 134) (Fig. 2).....	22
Composite curve of blood pressure in the caffeine experiments (Fig. 3).....	23
Average curves of blood pressure under caffeine (Fig. 4).....	24
Effects of caffeine on low blood pressure (Fig. 5).....	26
Curves of heart-rate under caffeine, vagi divided: type I (Fig. 6).....	29
Curves of heart-rate under caffeine, vagi divided: type II (Fig. 7).....	29
Average curves of heart-rate, vagi divided (Fig. 8).....	30
Effects of caffeine on heart-rate (vagi intact) (Fig. 9).....	30
Caffeine on the Huerthle excursions (Fig. 10).....	35
Caffeine on vasomotor center (rate of flow of prepared organs) (Fig. 11).....	36
Effect of caffeine on respiration, Huerthle and mean blood pressure, and vasomotor center (Fig. 12).....	37
Curves of blood pressure and oncometer (Fig. 13).....	38
Effects of caffeine on the respiration (Fig. 14).....	39
Acute death under small doses of caffeine (Fig. 15).....	43
Death after large doses of caffeine (Fig. 16).....	44
Death after very large dose of caffeine (Fig. 17).....	45
Effect of caffeine on sciatic stimulation (Fig. 1).....	53
Effect of caffeine on splanchnic stimulation (Fig. 2).....	54
Effect of caffeine on epinephrin $\frac{1}{100}$ mg. (Fig. 3).....	56
Effect of caffeine on epinephrin, $\frac{1}{20}$ mg. (Fig. 4).....	57
Effects of caffeine on spleen-volume (upper tracing), and mean blood pressure (Experiment C, 134) (Fig. 1).....	67
Caffeine 40 mg. per kg., intravenous (Experiment C, 109-4, vagi intact) (Fig. 2).....	68
Caffeine (10 mg.) on oncometer (middle tracing) and blood pressure (lower tracing) (Fig. 3).....	69
Caffeine 20 mg. on kidney oncometer (upper tracing) and blood pressure (second tracing) (Fig. 4).....	70
Effects of caffeine on respiration and blood pressure (Experiment C, 110-1) (Fig. 5).....	71
Intravenous injection of caffeine after large doses have been administered (Experiment C, 13-43) (Fig. 6).....	77
Caffeine on spleen oncometer (upper line) and blood pressure (Fig. 7).....	78
Diagram of the acute oncometer and blood pressure phenomena, with caffeine injection (Fig. 8).....	87
Segment of rabbit's intestine beating in defibrinated blood of epinephrectomized rabbit (Fig. 1).....	99
Rabbit's intestine in Ringer's solution (Fig. 2).....	99

Tracing from cardioplethysmograph, piston recorder (Fig. 1).....	118
Tracing from cardioplethysmograph (Fig. 2).....	119
Cat, volume of loop of intestine, piston recorder, etc. (Fig. 3).....	120
Cat, the splanchnics had been cut (Fig. 4).....	120
At 3.11, 5 cc. $\frac{N}{20}$ NaOH injected into saphenous vein (Fig. 5).....	125
Cat, male, 2100 g. ether, vagi intact (Fig. 6).....	126
At 3.33 p.m. 5 cc. $\frac{N}{20}$ sodium iodosobenzoate injected into saphenous vein (Fig. 7).....	126
At 3.41 p.m. 5 cc. $\frac{N}{20}$ sodium iodbenzoate injected into saphenous vein (Fig. 8).....	127
Female cat, 3.52 k. (See experiment 2) (Fig. 1).....	142
At 10, 2 cc. $\frac{N}{5.4}$ hydrocyanic injected into right femoral vein (Fig. 2).....	143
At 12, 1 cc. $\frac{N}{20}$ sodium iodosobenzoate injected into left femoral vein (Fig. 3).....	144
Effect of phenolphthalein oxime upon pyrexia in the rabbit (Fig. 1).....	172
Effect of phenolphthalein oxime upon pyrexia in the rabbit (Fig. 2).....	173
Cat, brain and cord pithed (Fig. 1).....	211
Continuation of experiment shown in Fig. 1 (Fig. 2).....	211
Cat, paraldehyde. Carotid blood-pressure (Fig. 3).....	213
Cat, brain pithed (Fig. 4).....	214
Isolated loop of rabbit's small intestine, etc. (Fig. 5).....	216
Continuation of record shown in Fig. 5 (Fig. 6).....	216
Alcohol on temperature (Fig. 1).....	274
Showing the effect of horse serum upon the heart and blood pressure of cat (Fig. 1).....	303
Showing the effect of horse serum upon the intrapleural pressure and upon the blood pressure of the carotid artery (Fig. 2).....	305
The effect of horse serum upon the heart and blood pressure of a cat treated with atropin sulphate (Fig. 3).....	308
Perfusion of blood-vessels of B. agua with Bufo-epinephrin at a pressure of 30 cm (Fig. 1).....	332
Action of bufagin on excised frog's heart (Fig. 2).....	349
Action of bufagin on excised heart of Bufo agua (Fig. 3).....	352
Perfusion of B. agua with bufagin (Fig. 4).....	354
Excised stomach of B. agua (Fig. 5).....	355
Parts of a graphic record illustrating action on circulation (Fig. 6).....	360
Dog (7 kilos). Ether. Showing effect of 2.1 mg. of bufagin in divided doses intravenously (Fig. 7).....	365
Dog (7 kilos). Ether (Fig. 8).....	368
Effect of bufagin on plain muscle (Fig. 9).....	370
Dog, (9.15 kilos). Trichlor-tertiary-butyl alcohol (Fig. 10).....	372
Dog (9.5 kilos). Ether and trichlor-tertiary-butyl alcohol (Fig. 11).....	372
Dog (6 kilos). Anaesthetized with trichlor-tertiary-butyl alcohol (Fig. 12).....	373
Cat (1.6 kilos). Anesthesia with trichlor-tertiary-butyl alcohol. Fig. 13).....	374
Cat (2.7 kilos). Trichlor-tertiary-butyl alcohol (Fig. 14).....	376
Intestinal strip beating first in Ringer's solution (Fig. 1).....	381
Intestinal strip beating in about 3.5 cc. normal defibrinated blood (Fig. 2).....	383
Intestinal strip beating first in Ringer's solution (Fig. 3).....	384
Absorption of sodium iodide according to time of sojourn (Fig. 1).....	392
The permeability of sodium iodide through the excised intestine, etc. (Fig. 2).....	408

The effect of various substances on the permeability of sodium iodide through the excised intestine (Fig. 3).....	409
Absorption of sodium iodide according to time of sojourn in hyperchloric animals (Fig. 4).....	417
Average curves of absorption of iodide in normal, chlorine-poor and hyperchloric animals (Fig. 5).....	417
Absorption of sodium iodide by animals whose tissues were poor in chlorides (Fig. 6).....	419
Kidneys of a nephritic, glycosuric and polyuric animal before the use of an anesthetic (Figs. 1 and 2).....	439
Kidneys of two animals which were excessively polyuric before the administration of an anesthetic (Figs. 3 and 4).....	439
Kidneys of two animals which were responsive to the diuretics (Figs. 5 and 6).....	439
Myocardiogram of turtle's heart with 1 per cent isocalycanthine hydrochloride irrigation (Tracing 1).....	446
Blood pressure and myocardiogram from cat with isocalycanthine hydrochloride injection (Tracing 2).....	448
Blood pressure and myocardiogram from cat with isocalycanthine hydrochloride and epinephrine injection (Tracing 3).....	450
Kidney volume, respiratory and blood pressure tracings from a dog (Fig. 1)...	494
Kidney volume, leg volume, carotid pressure and respiratory tracings from a dog (Fig. 2).....	497
Kidney volume, uterus, spleen volume, right carotid pressure and respiratory tracings from a dog (Fig. 3).....	498
Kidney volume, blood pressure and respiratory tracings from a normal etherized dog (Fig. 4).....	500
Kidney volume, uterus, time in seconds (Fig. 5).....	502
Volume of an excised perfused spleen from a dog (Fig. 6).....	505
Heart tracing showing action of epinephrine on strychnine depressed heart...	522
Cardiotomized frogs that have each received the same quantity of acid fuchsin (Figs. 1, 2, 3).....	587
Acute change in cardiac volume. Decreased volume with small doses of caffeine—cardioplethysmogram (Fig. 1).....	611
Acute changes in cardiac volume—cardiac plethysmograph (Fig. 2).....	613
Myocardiogram—left ventricle (Fig. 3).....	618
Effect of iodothylin per jugular upon amount of epinephrin in the blood (Fig. 1).....	628
Effect of infundibulin (20 per cent extract of the posterior part of the pituitary) upon the amount of epinephrin in the blood (Fig. 2).....	629
Effect of 0.118 cc. of egg albumen upon the amount of epinephrin in the blood thirty minutes after the injection per jugular (Fig. 3).....	631
Effect of 0.0648 gram of peptone (Merck) upon the contents of epinephrin in the blood (Fig. 4).....	632
Cat, 2 kgm. Injection of 0.6 mgm. (0.3 cc.).....	652
Cat, 2.8 kgm. Injection of 2.4 mgm. (0.8 cc.).....	654
Cat, 2.8 kgm. Injection of 0.5 mgm. (0.5 cc.).....	656
Cat, 2.7 kgm. The animal had received 1 cc. $\frac{1}{2}$ per cent solution of atropine sulfate seven minutes earlier.....	657



THE EFFECTS OF PILOCARPINE AND ATROPINE UPON THE AMYLOLYTIC POWER AND COM- POSITION OF THE SALIVA

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A large amount of experimental work has been done to show the action of pilocarpine and atropine upon the salivary secretion. This work has had to do almost entirely with the effects of these drugs on the amount of saliva secreted, and the manner in which they produce these effects.

It is well known that pilocarpine in moderate doses produces a profuse secretion of saliva, while relatively small doses of atropine will inhibit the secretion entirely; the current theory being that the former stimulates, and that the latter paralyzes the secretory nerve endings (1), (2).

Cushny (3) found that it required only 0.5 mg. of atropine to inhibit the secretion of saliva caused by 5 mg. of pilocarpine, the ratio of strength being therefore about one part of atropine to ten parts of pilocarpine.

The effect of atropine upon the formation of the solid constituents of saliva is not so well known as its effect upon the volume secreted. Langley (4) states that after administration of atropine and then stimulation of the chorda, he never saw in microscopic sections of the gland any indication of the formation of fresh substance; whereas, without atropine a distinct outer protein zone would have been formed.

It is generally stated that pilocarpine increases the *total amount* of solids secreted, though not the *percentage composition*. Sollman (5), in speaking of the influence of pilocarpine upon secre-

tions in general, says "The total solids are also increased (with the volume), although their percentage is lessened."

In none of the experimental work done, has any attempt been made to show what changes occur in the amylolytic power after administration of atropine and pilocarpine, and in the composition of the saliva after administration of atropine. It seemed of interest to determine this, and the experiments to be reported were accordingly carried out.

EXPERIMENTAL

Technique of the Experiments

Collection of Saliva. The reflex secretion of saliva was excited by chewing ordinary paraffine. The collection was made in the morning before breakfast, so that the glands would be in an approximately constant condition of rest before the period of secretion. Dr. R. B. Gibson alternated with me in collecting the saliva for the experiments. The results show the variations to be practically the same for the two individuals.

It was intended to collect normal samples for several days, and then samples for several days showing the drug effects, and average the two series for comparison. Table 1 gives some results obtained with this method. The normal samples, however, showed such great variations from day to day that it was decided that no definite conclusions could be arrived at by this method. In the remaining experiments the saliva was collected in separate samples for consecutive fifteen minute periods, during the entire time of the secretion, which was usually of about an hour and a half to two hours duration. Six or eight samples collected in this way served as a control, and also furnished interesting results concerning the normal variations in saliva during a continuous period of secretion.

For the drug experiments, normal saliva was collected for the first fifteen minute period as the control, a gelatin capsule containing the pilocarpine or atropine was then swallowed and the consecutive collections of samples which would show the varia-

tions by the drug then made. In this way the time of the maximal and minimal effects of the drug could be determined; and by collecting the first sample as a control, any error due to change of the normal standard for amylolytic power and solid content during the last twenty-four hours could be eliminated.

Determination of the Amylolytic Power. In determining the amylolytic power of the various samples of saliva, 5 cc. of filtered saliva were added to 150 cc. of 2 per cent starch paste prepared from pure arrow-root starch; the digestion was continued at room temperature for twenty minutes and was then stopped by the addition of 50 cc. of 20 per cent sodium carbonate solution. This method of stopping the digestion with strong alkali solution is an improvement over the usual method of boiling the mixture; it is much more convenient, and the exact time of digestion is much more accurately controlled.

The amount of maltose produced was then determined by Benedict's (11) method. As Benedict's formula was for dextrose, control titrations were made with 1 per cent maltose solution, and it was determined that the 10 cc. of copper sulphate solution was equivalent to approximately 0.115 gram of maltose, the equation for determining the amount of maltose produced being:

$$y \text{ Titration} : 0.115 \text{ g} = 100 : x$$

Determination of Total Solids and Ash. For the determination of the content in total solids, the standard method of drying in low crucibles on the water bath and then in the air bath at 105°, was employed. The residue was weighed for the total solids then ashed and weighed again to determine the content of inorganic solids. The difference between the two results thus obtained was taken as the content of organic constituents. A definite quantity, 5 cc. of filtered saliva, was used in all these determinations for the content in solids.

Control Experiments. The variations in table 1, in saliva collected for a fifteen minute period on different days, demonstrated the impossibility of using the normal results of one day as a control for accurate drug effects on the next. These variations,

though not great, were enough to eliminate any definite conclusions as to the constancy of normal saliva, or of the variations caused by the drugs. They serve, however, to illustrate the changes in the normal composition of saliva from day to day. Thus in samples within a period of three days, the volume had changed 14 cc., the amylolytic power 130 mg. of maltose produced, and the content in total solids 0.08 per cent.

TABLE 1

Normal variations in saliva. Fifteen minute collections over a period of seven days

Date of experiment . . .	1-7-09	1-10	1-11	1-12	1-13	1-13	1-14	1-17	1-20
Subject	E	E	E	E	E	G	G	G	G
Volume cubic centimeters	16	21	20	21	30	21	35	30	27
Amylolytic power; grams maltose	0.718	0.676	0.718	0.575	0.580	0.851	0.234	0.469	0.605
Total solids per cent . . .	0.46	0.54	0.52	0.48	0.54	0.46	0.56	0.54	0.58
Organic solids per cent . .	0.34	0.48	0.40	0.36	0.38	0.38	0.50	0.40	0.48
Inorganic solids per cent	0.12	0.06	0.12	0.12	0.16	0.08	0.06	0.14	0.10

The results obtained from the saliva collected in consecutive fifteen minute periods, however, showed very little variation throughout the period of secretion.¹ In table 2 it is seen that the *volume* of saliva secreted per fifteen minute period, is approximately constant for the six consecutive periods. Although there are slight increases and decreases in some of the experiments, I have found that these changes tend to coincide with variations in the strength with which the paraffine was chewed, and that even at the last of the longest experiments I could always increase the volume slightly by additional stimulation of the glands in this way.

Content in Solids. In making up the tables I have computed not only the normal variations in the percentage composition of

¹ Nine experiments were performed to show normal variations, variations after pilocarpine and variations after atropine, but to avoid making the tables cumbersome, the results of only three experiments from each of the series have been given in tables 2, 3 and 5. The remaining experiments showed the same variations.

TABLE 2

Variations in normal saliva. Samples collected in consecutive 15 minute periods. In this and the following tables, the solids for each period (in grams) and the quantity per 100 cubic centimeters of saliva are given as "Amount" and "Per cent," respectively

EXPERIMENT AND COLLECTOR	CONSECUTIVE 15-MINUTE PERIODS	VOLUME	AMYLOLYTIC POWER GRAMS MALT-TOSE PRODUCED	TOTAL SOLIDS AMOUNT AND PERCENTAGE	ORGANIC SOLIDS	INORGANIC SOLIDS
		cc.	grams			
4. G.....	1	45	0.766	0.297 gms. 0.66 %	0.207 gms. 0.467 %	0.09 gms. 0.20 %
	2	40	0.756	0.264 0.66	0.176 0.44	0.08 0.22
	3	43	0.746	0.275 0.64	0.197 0.46	0.07 0.18
	4	40	0.718	0.248 0.62	0.168 0.42	0.08 0.20
	5	40	0.718	0.224 0.56	0.144 0.36	0.08 0.20
	6	43	0.741	0.258 0.60	0.163 0.38	0.09 0.22
7. E.....	1	35	0.500	0.152 0.38	0.120 0.30	0.032 0.08
	2	30	0.479	0.102 0.34	0.078 0.26	0.024 0.08
	3	30	0.500	0.102 0.34	0.078 0.26	0.024 0.08
	4	30	0.479	0.102 0.34	0.078 0.26	0.024 0.08
	5	30	0.500	0.102 0.34	0.078 0.26	0.024 0.08
	6	33	0.522	0.112 0.34	0.085 0.26	0.026 0.08
9. E.....	1	25	0.756	0.100 0.40	0.70 0.28	0.03 0.12
	2	25	0.766	0.100 0.40	0.70 0.28	0.03 0.12
	3	25	0.766	0.100 0.40	0.70 0.28	0.03 0.12
	4	25	0.766	0.100 0.40	0.70 0.28	0.03 0.12
	5	25	0.766	0.100 0.40	0.70 0.28	0.03 0.12
	6	35	0.766	0.154 0.44	0.119 0.34	0.035 0.10

the solid content, but also the actual amount of solids secreted during each of the entire fifteen minute periods. So in the case of the volume of the saliva secreted, both the amount and percentage of total solids, seem to remain practically the same throughout the experiments (table 2). Any change that occurs, is a very slight diminishing of both amount and percentage composition after the first period of secretion. Practically the same can be said of the amount and percentage of organic solids. The amount and percentage of inorganic solids is constant, in the successive periods (table 2). The slight falling off of total solids is due, then, to the change occurring in the organic solid content.

Amylolytic Power. The amylolytic power also remains approximately constant throughout the periods of secretion (table 2). There may be a very slight falling off during one or two of the fifteen minute periods, but the amount of maltose produced is usually just as great for the last period as for the first.

Summary of Control Experiments. From the control observations it seems, therefore, that the results obtained from a series of samples collected in consecutive fifteen minute periods, would serve for comparison with the drug variations. Such slight changes as did occur, were entirely in accordance with the literature—that the volume of saliva secreted varies to some extent with the strength of stimulus (6), and that there is a decrease in the per cent of organic solids over a continuous period of secretion (7). The diminution of organic solids in my experiments, however, was not a gradual decrease such as Carlson and McLean obtained with the cat, but occurred in most cases about the second fifteen minute period of secretion, the amount and percentage composition usually being normal during the fifth and sixth periods. It is apparent, then, that there is not sufficient variation in the character of the normal saliva in the consecutive periods to need special consideration for comparison with the decided effects obtained after the administration of pilocarpine and atropine.

Chittenden and Richards (8) showed that the amylolytic power of human saliva varied throughout the day, it being greater

before meals than afterward. It must be remembered therefore that all of the present experiments were performed when the secretion of the ptyalin would be at its maximum, *i.e.*, before breakfast.

VARIATION IN THE COMPOSITION AND AMYLOLYTIC POWER OF SALIVA AFTER THE ADMINISTRATION OF PILOCARPINE

Results Given in Table 3

Volume. The well known effect of pilocarpine upon the volume secreted is shown and need not be discussed further except to mention that the maximal increase occurred usually in the third period in the case of the smaller doses. After the larger doses the volume remained high until the experiment was concluded.

Content in Solids. The results (table 3) show that pilocarpine largely increases the actual amount of total solids secreted, but that the *percentage* of total solids remains more nearly normal. From the percentage variations, it seems that if there is any change in the percentage composition for the total solids, it is rather an increase, and not a decrease as stated by Sollman. For example, in experiment 7, the saliva secreted during the control period contained 0.163 gram of total solid, or 0.44 per cent; following the administration of 10 mg. of pilocarpine, the figures for the six consecutive fifteen minute periods were 0.150 gram and 0.50 per cent; 0.264 gram and 0.48 per cent; 0.350 gram and 0.50 per cent; 0.276 gram and 0.46 per cent; 0.208 gram and 0.40 per cent; and 0.180 gram and 0.40 per cent. The maximum effect upon the total solid in this experiment, then, occurs in the third period after the administration of the drug and is coincident with the maximum increase in the volume secreted. In the last two periods, the percentage content fell slightly below the normal of the control period. In experiment 4, however, the per cent of total solid remained constant throughout the successive periods, the volume secreted having increased from 25 cc. in the control fifteen minutes to 43 cc. in the second period after the administration of the pilocarpine.

TABLE 3

Variations in the saliva after administration of pilocarpine. Samples collected in consecutive fifteen-minute periods; drug taken between first and second periods

EXPERIMENT AND COLLECTOR	CONSECUTIVE 15-MINUTE PERIODS	VOLUME	AMYLOLYTIC POWER GRAMS MALTOSE PRODUCED	TOTAL SOLIDS AMOUNT AND PERCENTAGE	ORGANIC SOLIDS	INORGANIC SOLIDS
		cc.	grams			
4. E	1	25	0.821	0.110 gms.	0.090 gms.	0.02 gms.
5 mgs. pilo-	(control)			0.44%	0.36%	0.6 %
carpine	2	25	0.493	0.110	0.09	0.02
taken be-				0.44	0.36	0.08
tween first	3	43	0.821	0.189	0.154	0.034
and sec-				0.44	0.36	0.08
ond pe-	4	41	0.821	0.180	0.147	0.032
riods.				0.44	0.36	0.08
	5	35	0.821	0.154	0.133	0.021
				0.44	0.38	0.06
6. G	1	37	0.676	0.222	0.162	0.059
10 mgs.	(control)			0.60	0.44	0.16
pilo-	2	40	0.676	0.256	0.184	0.072
carpine				0.64	0.46	0.18
.....	3	54	0.676	0.345	0.237	0.108
				0.64	0.44	0.20
	4	55	0.310	0.363	0.253	0.110
				0.66	0.46	0.20
	5	563	0.310	0.328	0.233	0.095
				0.62	0.44	0.18
	6	65	0.319	0.416	0.299	0.117
				0.64	0.46	0.18
	7	45	0.287	0.315	0.189	0.126
				0.70	0.42	0.28
7. E.	1	37	0.442	0.163	0.111	0.051
10 mgs.	(control)			0.44	0.30	0.14
pilo-	2	30	0.370	0.150	0.108	0.042
carpine				0.50	0.36	0.14
.....	3	55	0.348	0.264	0.187	0.077
				0.48	0.34	0.14
	4	70	0.359	0.350	0.225	0.098
				0.50	0.36	0.14
	5	60	0.383	0.276	0.192	0.084
				0.46	0.32	0.14
	6	52	0.323	0.208	0.135	0.072
				0.40	0.26	0.14
	7	45	0.255	0.180	0.117	0.053
				0.40	0.26	0.14

By reference to the figures for the organic and inorganic solids it will seem that these changes, *i.e.*, an increase in amount and percentage of total solids, are due to an increase in amount and percentage of both the organic and the inorganic constituents. The greatest variation, however, appears to occur in the amount of the organic solids secreted. These results indicate that the "trophic" as well as the "secretory" fibers, or cell elements, are stimulated to increased activity by pilocarpine.

Amylolytic Power. The amylolytic power of the saliva is very materially diminished by pilocarpine, a decrease occurring in every experiment performed in spite of the fact that the content in solids is maintained. There is no definite relation between the quantity of the drug taken and the decrease in amount of maltose produced by 5 cc. of the saliva employed. For instance, in experiment 4 with a dose of 5 mg. of pilocarpine there was a decrease of 328 mg. of maltose—in experiment 6 with 10 mg. of pilocarpine there was a decrease of 366 mg. of maltose—while in experiment 8 after a dose of 13 mg. of the drug, there was only a decrease of 129 mg. of maltose produced. Furthermore, there seems to be no constant relation between the volume secreted and the amylolytic power. For example, in experiment 5, the normal volume and amylolytic power were 19 cc. and 766 mg. of maltose, respectively, while in the fourth period of this experiment the volume secreted was 50 cc. Yet the amylolytic power was again normal, *i.e.*, 766 mg. of maltose were obtained.

In the preceding tables and discussion, only the relative amylolytic power of the saliva after administration of atropine or pilocarpine, as compared with that of the normal secretion, has been considered.

The same amount of saliva, 5 cc., was used in each digestion and the amount of maltose produced determined. As has been shown, the changes were very great, the results indicating that either the activity of the ptyalin was diminished, or what is more probable, that the actual amount of the enzyme secreted was decreased.

Besides the question of relative amylolytic power, *i.e.*, the amounts of maltose produced by the same quantities of saliva—

there is another point to be considered. In the case of a drug like pilocarpine, which increases the volume of saliva secreted to such an enormous extent, would not the amount of maltose produced by the total volume secreted in a given period be greater than that produced by the normal saliva secreted in the same length of time, although the relative amylolytic power of the drug saliva is less than that of the normal secretion? To determine this point, I calculated the amounts of maltose produced by the total volumes of saliva secreted in the various fifteen minute periods after administration of pilocarpine, and compared the results with the maltose produced by the total volumes of the normal saliva secreted in the control periods. The figures in table 3 are used as a basis for the calculations. In interpreting the results, it should be recalled, of course, that the activity of the ptyalin may not be increased in the same ratio as its concentration.

The results in table 4 show that the total amount of ptyalin, as measured by the quantity of maltose produced by the total volumes of the saliva secreted in the various fifteen minute periods, is greater after pilocarpine than the normal saliva. In other words, although there is less ptyalin in a given quantity of saliva after administration of the drug, than in the same amount of

TABLE 4

Variations in actual amount of maltose produced by the total volumes of saliva secreted after administration of pilocarpine. The figures represent the amounts of maltose which would be produced by the total volume of saliva collected in the various 15 minute periods. The results in table 3 were used as a basis for calculation

EXPERIMENT AND COLLECTOR	GRAMS MALTOSE PRODUCED BY TOTAL VOLUMES OF SALIVA COLLECTED IN THE RESPECTIVE 15-MINUTE PERIODS							
	CONTROL	PILOCARPINE	Period 2	Period 2	Period 4	Period 5	Period 6	Period 7
	Period 1							
4. E	4.10	mg. 5	2.46	7.06	6.73	5.74		
5. E	2.91	10	4.23	4.59	7.18	7.66		
6. G	5.00	10	5.40	7.30	3.41	3.28	2.29	2.57
7. E	3.27	10	2.22	3.82	5.02	4.59	3.55	2.29
8. E	4.05	13	3.28	6.44	8.62	7.01	7.52	

normal saliva, the increased volume of the secretion caused by the drug, more than compensates the diminished formation or secretion of the enzyme. Of course, unless the effect upon the volume is well marked, this compensation does not occur.

Summary to the Effects of Pilocarpine

Pilocarpine, then, in doses of 5 to 13 mg., largely increases the total amount of solids secreted along the well known effect upon volume. The percentage content of total solids, however, is not diminished; on the contrary, the concentration is often increased somewhat, and this increase involves especially the organic constituents. The amylolytic power of the saliva is very materially diminished in all the experiments performed with pilocarpine. There is no constant relation observable between the amylolytic properties of the saliva and the amount of pilocarpine taken or the volume of the secretion. Because of the increased volume, however, the total amount of ptyalin secreted after pilocarpine is greater than that normally obtained. Inasmuch as the amylolytic powers of the saliva are markedly lessened, while the percentage content in total solids is maintained or increased after pilocarpine, an independently controlled process is suggested for the elaboration of the ptyalin.

VARIATIONS IN THE COMPOSITION AND AMYLOLYTIC POWER OF THE SALIVA AFTER THE ADMINISTRATION OF ATROPINE

The same methods were used with the atropine experiments as in the case of pilocarpine—a normal sample was collected during the first fifteen minute period, the atropine administered, and the collection continued.

Results Given in Table 5

Volume. The usual decrease in the volume of saliva secreted was obtained, the maximal effect of the atropine occurring toward the end of the experiments. In some of the experiments there was not enough saliva secreted in the sixth and seventh periods for analysis.

TABLE 5
Variations in the saliva after administration of atropine

EXPERIMENT AND COLLECTOR	CONSECUTIVE 15 MINUTE PERIODS	VOLUME	AMYLOLYTIC POWER GRAMS MALTOSE PRODUCED	TOTAL SOLIDS AMOUNT AND PERCENTAGE	ORGANIC SOLIDS	INORGANIC SOLIDS
		cc.	grams			
1. E 0.5 mg. atropine taken between first and second periods.....	1	38	0.500	0.182 gms. 0.48%	0.136 gms. 0.36%	0.045 gms. 0.12%
	(control)					
	2	32	0.410	0.128 0.40	0.089 0.28	0.038 0.12
	3	35	0.442	0.140 0.40	0.112 0.32	0.028 0.08
	4	32	0.425	0.128 0.40	0.089 0.28	0.038 0.12
	5	32	0.410	0.128 0.40	0.089 0.28	0.038 0.12
	6	26	0.495	0.109 0.42	0.078 0.30	0.031 0.12
	1	55	0.460	0.363 0.66	0.286 0.52	0.077 0.14
	(control)					
	2	55	0.315	0.308 0.56	0.220 0.40	0.088 0.16
	3	50	0.323	0.280 0.56	0.200 0.40	0.080 0.16
	4	50	0.359	0.260 0.52	0.180 0.36	0.080 0.16
4. G 0.5 mg. atropine....	5	40	0.302	0.250 0.50	0.128 0.32	0.072 0.18
	6	25	0.277	0.120 0.44	0.085 0.30	0.035 0.14
	7	25	0.359	0.120 0.44	0.065 0.26	0.040 0.16
	1	40	0.575	0.26 0.66	0.192 0.48	0.072 0.18
	(control)					
	2	40	0.575	0.264 0.66	0.184 0.46	0.080 0.20
	3	35	0.522	0.22 0.64	0.140 0.40	0.084 0.24
	4	30	0.460	0.174 0.58	0.114 0.38	0.060 0.20
	5	17	0.410	0.085 0.50	0.054 0.32	0.030 0.18
	6	15	0.389	0.072	0.045	0.27
	7	10		0.48	0.030	0.18
5. G 1 mg. atropine.....	1	40	0.575	0.26 0.66	0.192 0.48	0.072 0.18
	(control)					
	2	40	0.575	0.264 0.66	0.184 0.46	0.080 0.20
	3	35	0.522	0.22 0.64	0.140 0.40	0.084 0.24
	4	30	0.460	0.174 0.58	0.114 0.38	0.060 0.20
	5	17	0.410	0.085 0.50	0.054 0.32	0.030 0.18
	6	15	0.389	0.072	0.045	0.27
	7	10		0.48	0.030	0.18

Note. In the last two experiments the saliva collected in periods 6 and 7, because of the small volumes, was combined for analysis.

Content in Solids. There is a marked decrease in the *total* amount of the solids secreted after atropine, the quantity diminishing steadily as the experiment progresses (table 5). The percentage of the total solids in the saliva is not increased; there is, in fact, a tendency for the per cent of solids to be diminished and in two experiments (4 and 5), there is a distinct fall. For instance, in experiment 4, the total solids secreted for the control period were 0.363 gram in a concentration of 0.66 per cent; subsequent to the administration of 0.5 mg. of atropine, the figures fell for the successive periods as follows; 0.308 gram and 0.56 per cent; 0.280 gram and 0.56 per cent; 0.260 gram and 0.52 per cent; 0.200 gram and 0.53 per cent; and 0.120 gram and 0.54 per cent. In the same experiment, the volume for the content period was 55 cc., and in the following periods 55 cc., 50 cc., 50 cc., 40 cc. and 25 cc. It will be noted that lowering of the percentage content in solids is much more pronounced for subject G (experiments 4 and 5), whose saliva is characterized by a high normal content in solids than in the other experiments (subject E).

The figures for the variations in inorganic solids, show the percentage composition of the inorganic constituents to be fairly constant or even slightly increased, so that any decrease in the per cent of total solids must be due in large part to a diminishing of the organic constituents. This is shown where the amount and percentage of organic solids decrease along with the total solids. In experiments 4 and 5, the amount and percentage of organic constituents were still on the decline at the end of the period of secretion, while in some of the others the content is more nearly normal about the sixth period.

Amylolytic Power. The amylolytic power of the saliva is markedly diminished, after atropine, the maximal decrease usually occurring about the second period after administration of the drug. In the majority of the experiments there is a tendency for the amylolytic activity to increase towards the fifth or sixth periods, as though the drug effect had worn off. It is seen that (table 5) although the amylolytic power has returned to almost normal in the later periods, the volume secreted and the content in solids show that the drug is still acting.

Summary of Effects of Atropine

These experiments with atropine, then, show the well known decrease in the volume of the saliva secreted. Furthermore, the drug has an inhibiting influence upon the formation or secretion of the content in total solids and the ptyalin. Since the amylolytic powers are usually recovered while the volume and content in total solids is still influenced by the atropine, a selective action of the drug here, as with pilocarpine, is suggested.

To just what physiological action of the drug these variations are due, is a question to be determined. Barcroft (9) has shown that atropine exerts a marked influence upon the oxygen intake of the salivary glands; Matthews' (10) experiments on the starfish and sea-urchin embryos, point to the fact that atropine retards the oxidative decomposition of protoplasm. It seems probable, therefore, that the diminution which I have shown in the secretion of the saliva after atropine, is due to a retarding of the oxidative metabolic processes which take place within the secretory cells.

GENERAL DISCUSSION

Langley (1) and Marshall (2) have concluded that the antagonism of atropine and pilocarpine is physiological, *i.e.*, a question of affinity shown by the tissues for one drug or the other.

In applying Matthews' (10) results, showing that pilocarpine hastens, and that atropine retards, the development of embryos, to the secretion of saliva, it seems that any increase or decrease in activity of the salivary cells after pilocarpine or atropine, is due to an increase or decrease in the oxidative decomposition of the cell protoplasm.

Whether the nerve endings or the cells themselves, are acted upon by the drugs, is at present undecided, although most physiologists have accepted the former theory.

The results in the present paper are what would naturally be expected when working with two sets of drugs as pilocarpine and atropine. Atropine exerted an inhibitory influence upon the formation or activity of all the properties of the saliva which were

considered. Pilocarpine, with the exception of its effect upon the amylolytic power of the saliva, had a stimulating action upon the formation of these same components. It must be remembered that the conclusion that pilocarpine reduces the amylolytic power of saliva is the result of the comparison of the same amounts of normal saliva and pilocarpine saliva. It is, therefore, a reduction in the relative or percentage content of ptyalin. As has been stated, because of the great increase in volume after pilocarpine, the total quantity of ptyalin present, more than compensates for the percentage decrease due to dilution.

The fact that the two drugs have opposite effects upon the other properties of saliva, while both decrease the amylolytic power, though to a different degree, brings out the point already mentioned—that the secretion of ptyalin seems to be entirely independent of the formation of any other components of the saliva.

I have pointed out that the amylolytic power has no definite relation to the volume secreted. The additional fact that the amylolytic activity is diminished, both when the solids are increased and when decreased, justifies the conclusion that the formation of ptyalin is independent of the other salivary constituents.

The action of atropine and pilocarpine upon the amylolytic power seems also to be of different duration from the effect upon the other constituents of the saliva. In many of the experiments with both drugs, the amount of maltose produced has again reached the level at a time when the volume and percentage of solids still show great variations.

In these experiments, only therapeutic doses of the pilocarpine and atropine were taken, but as the smallest and largest doses causes practically the same variations, the results may be considered as characteristic of the physiological effects of the two drugs.

CONCLUSIONS

1. The volume of the normal saliva, its amylolytic power, and the amount and percentage composition of solids secreted, remain approximately constant during a continuous period of secretion of six or eight fifteen minute periods. If there is any change, it is a very slight falling off of the percentage composition of organic solids, and at times, of the amylolytic power.

2. Pilocarpine reduces the relative amylolytic power of the normal saliva from 30 to 60 per cent. Although the relative amylolytic power of the saliva is much diminished by pilocarpine, the amount of maltose produced in the total volume of saliva secreted in a given period, after the administration of this drug, is greater than that produced by the amount of normal saliva secreted in the same length of time. "The efficiency" of the secretion is therefore increased by pilocarpine.

3. Pilocarpine increases the amount and tends to increase the percentage of both the organic and inorganic solids of the saliva; the greatest increase is in the organic constituents, however. Any percentage increase of the solids is not nearly so great as the increase in the actual amount secreted.

4. Atropine diminishes the amylolytic power of the saliva from 15 to 30 per cent.

5. Both the amount and percentage composition of total solids secreted are greatly diminished by atropine. The decrease is in the organic constituents.

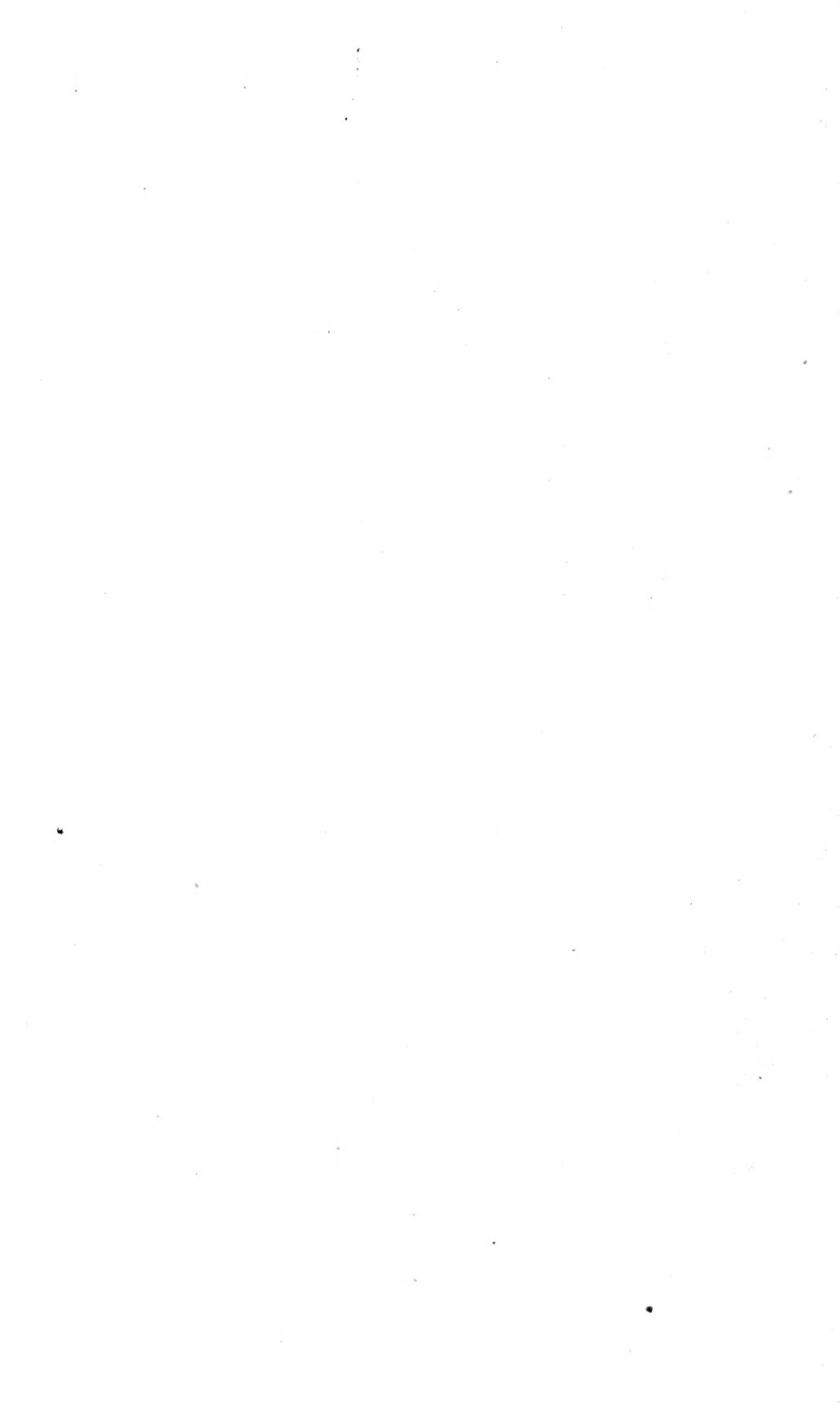
6. Pilocarpine and atropine affect the factors which influence both the "trophic" and "secretory" elements of the saliva.

7. The effect of pilocarpine and atropine upon the secretion, or activity of the ptyalin of the saliva, bears no definite relation to the action of these drugs upon the other physico-chemical properties of the secretion.

I desire to thank Dr. R. B. Gibson who has aided and directed me in my work.

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THE ACTIONS OF CAFFEIN ON THE MAMMALIAN CIRCULATION

I. THE PERSISTENT EFFECTS OF CAFFEIN ON THE CIRCULATION

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CONTENTS

Introduction.....	19
Permanent effects on the blood pressure.....	22
Effects of caffein on the heart rate.....	28
Persistent effects on the vasomotor center.....	35
Persistent effects on the organ volume.....	37
Effects on respiration.....	40
Convulsive phenomena.....	40
Fatality.....	41
Summary.....	47

INTRODUCTION

The effects of caffein on the circulation have considerable interest, practical as well as theoretical. They have, accordingly been the subject of numerous investigations. Unfortunately—as is so often the case—the results and conclusion of the investigators have been far from uniform—in fact, they appear to be flatly contradictory. Our own researches were undertaken in the hope that the confusion might be cleared away by a somewhat more extensive study, comprising a sufficient number and especially a sufficient variety of experiments. This hope has been

¹This investigation was started at the request of the Bureau of Chemistry of the Department of Agriculture of the United States.

largely realized, we believe. Our results show that the actions of caffeine are rather complex, and therefore subject to considerable variation according to experimental conditions, and these conditions account fully for the existing contradictions. These factors may be analyzed, and their proper interpretation clears up the entire subject. When they are understood, it is easy to reproduce all the apparently contradictory results which have been recorded.

Our experiments were made mainly on dogs, under morphine-anesthesia (20 mg. per kg.), supplemented by ether during the operation, and generally by curare. Some cats were also used, with morphine-atropin-ethylcarbamate narcosis. The present report covers the records of about three hundred injections, made on twenty-nine animals. The injections were always made intravenously (generally into the femoral vein), using a 1 per cent solution of free caffeine in normal saline solution. *The doses throughout these papers must be understood as milligrams of free caffeine per kilogram bodyweight.*

Two of the most important complicating factors are, naturally, the dosage of the drug, and the time of its sojourn in the circulation. In order to appreciate these factors, we followed the general plan of making successive injections (into the femoral vein) of 2, then 3, then 5, 10, 20, etc., mg. of caffeine (per kilo of bodyweight) waiting between the injections until the phenomena had settled down to a constant. In this way, two series of events could be studied: 1, the immediate effects of single injections, and their modification by repeated injections; and 2, the more sustained effects of increasing doses.

We shall first present the study of these sustained effects since they are perhaps more characteristic of caffeine, and are of greater practical importance. For the sake of clearness, however, it may be well to outline the main acute effects briefly in this place (see figs. 1 and 2):

As the injection enters the vein, there is a slight (cardiac) rise of blood pressure, of 5 or 10 mm. Immediately after this, the pressure falls sharply, and then recovers almost as suddenly. The final level of pressure—which may be above or below the

normal—is thus attained in a very few minutes, but when the injections were repeated, at least ten minutes were generally allowed for this adjustment.

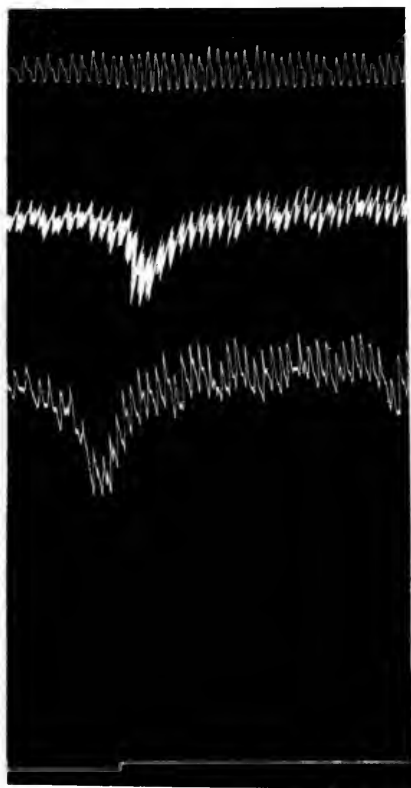


FIG. 1. EFFECTS OF CAFFEIN ON RESPIRATION AND BLOOD PRESSURE (EXPERIMENT C, 110-1)

Injection of 16.5 mg. of caffein per kg. (vagi divided). *Upper tracing*, respiration from tambour connected with trachea, with open side-tube. *Middle tracing*, carotid pressure tracing by Harvard membrane manometer. *Lower tracing* mean pressure by damped mercury manometer. The signal line in all the figures represents zero pressure of the mercurial manometer.

All the tracings are reduced to about $\frac{2}{3}$ natural size. They read from left to right, the drum moving about 17 mm. per minute.

PERMANENT EFFECTS ON THE BLOOD PRESSURE

The blood pressure data were plotted from the tracings. Fig. 3 presents a composite of these curves from all the caffein experiments. It will be seen that the effects are surprisingly uniform. This is brought out still more strikingly by the average curves shown in fig. 4.

Experiments starting with normal blood pressure (fig. 4, Curves I a and b): With doses of caffein up to 20 mg. the perma-

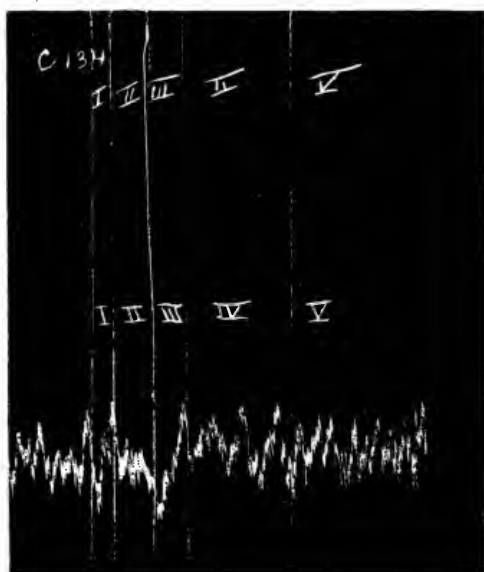


FIG. 2. EFFECTS OF CAFFEIN ON SPLEEN-VOLUME (UPPER TRACING), AND MEAN BLOOD PRESSURE (EXPERIMENT C, 134)

Ten milligrams of caffein after a preceding injection of 10 mg. (The Roman numerals indicate the successive stages of the action, as explained in Paper V).

nent effects are rather variable, but generally small (fig. 3). Somewhat less than half the experiments show a slight permanent rise, usually less than 15 mm. In the most of the remaining experiments the pressure remains about level, but a few show a fairly severe fall, so that the means of all the experiments show a fall of about 5 mm. (fig. 4). When the dose exceeds 20 mg. the

pressure begins to decline progressively with each additional dose. The fall is fairly rapid, until the pressure reaches the level of 50 to 70 mm., requiring a dosage of 125 to 300 mm. The blood pressure then remains almost constant, notwithstanding further

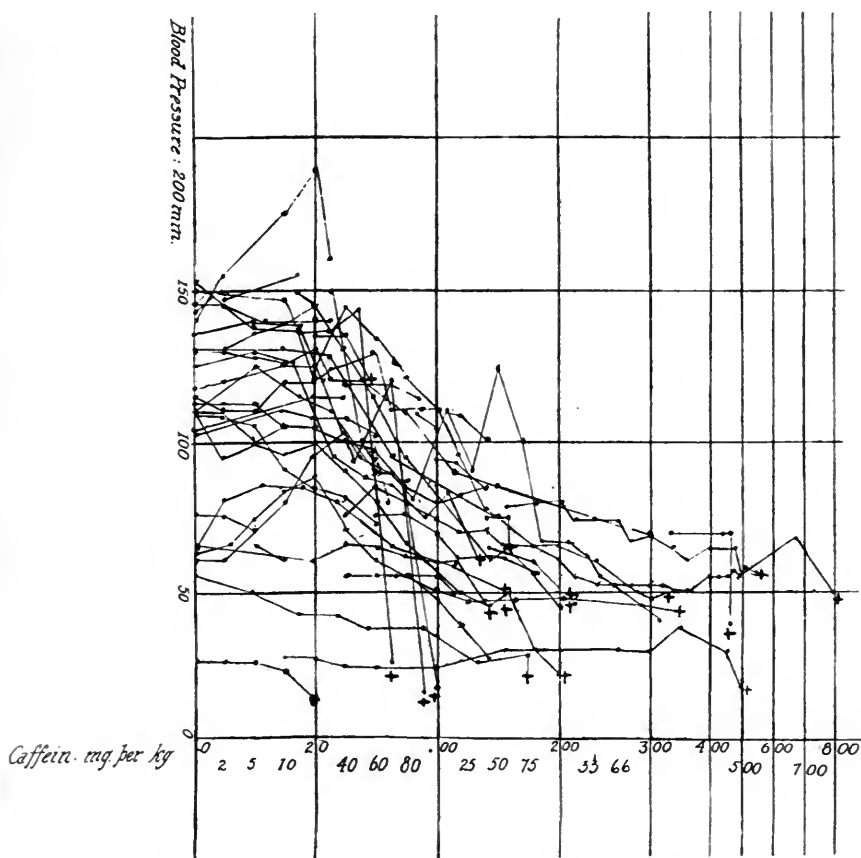


FIG. 3. COMPOSITE CURVE OF BLOOD PRESSURE IN THE CAFFEIN EXPERIMENTS
+ denotes that the animal died after the injection.

doses of caffeine (even up to 800 mg. per kg.) until the approach of death, when there is a sudden further fall. In uncomplicated experiments a fall of pressure below 50 mm. generally means that the next dose will be fatal.

In the majority of these experiments, both vagi were divided before the caffeine was injected. Many of the animals were also under the influence of curare. All the curves, however, correspond closely to the average. Four experiments were made on cats, and these also show the same phenomena.

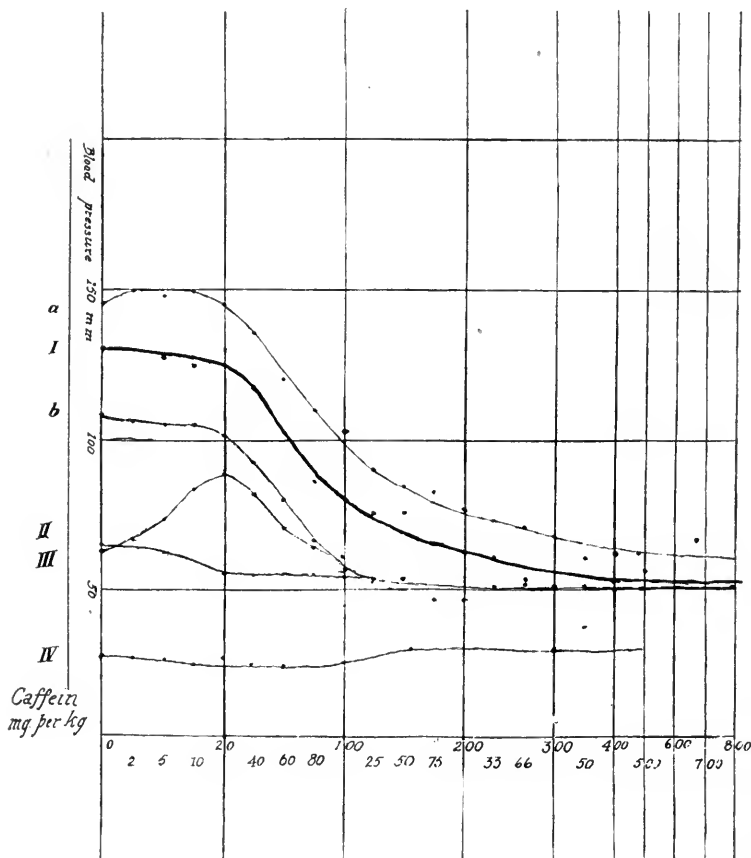


FIG. 4. AVERAGE CURVES OF BLOOD PRESSURE UNDER CAFFEIN

Curve I (heavy line) presents the mean height of blood pressure under caffeine for all experiments in which the original pressure was normal, i.e., between 100 and 150 mm. Curve a shows the upper range and curve b the lower range, in these experiments, after eliminating about 10 per cent of the extreme numbers in both directions. With low initial blood pressures, two distinct types of curves occur, shown as II and III. The curve when the initial pressure is at "shock" level is shown in IV.

There are therefore two critical points in the caffein curve: The first corresponding to the start of the fall of blood pressure, generally with doses of 20 mg.; the second, when the pressure reaches the level of 50 to 70 mm., generally corresponding to a dosage of about 150 mg. per kg., but largely independent of the dose.

Persistent rise of blood pressure under caffein. Whilst doses of caffein above 20 mg. per kg. lower the general blood pressure markedly, and fairly constantly, smaller doses in general tend to produce a slight rise (see figs. 1 and 2).

The records of 27 animals show that:

In sixteen the blood pressure did not rise permanently, at least not more than 5 mm. In eleven there was some rise. In eight of these, the rise was only between 8–20 mm., averaging $13\frac{1}{2}$ mm. In three, the rise was more marked, but according to the records, these are evidently explained by accidental causes or indirect actions, such as convulsive phenomena; or the onset of recovery from asphyxia. They are so exceptional, that they do not merit further discussion.

Experiments with low initial blood pressure. These have been included in the general summary, but deserve additional separate discussion. Reference to figs. 3 and 4 will show that they tend to three distinct types. These may be better understood by the explanation of the individual experiments, as plotted in fig. 5.

In Experiment C, 123, the vasomotor center was destroyed by section and pithing; there was considerable hemorrhage. The caffein caused no rise (except the mechanical rise at the moment of injection); on the contrary, the blood pressure, fell steadily from 55 to 30 mm. (total dose, 175 mg.) when death occurred by cardiac paralysis.

In the other seven experiments, the low pressures occurred spontaneously, apparently through partial asphyxia. In two of these (C, 136 and 137), the course is the same (type III) as with intentional destruction of the vasomotor center, the pressure remaining about the 50 mm. level. In three others (C, 115, 128 and 145) the pressure recovered partly under the smaller doses of caffein, to follow the usual curve under the influence of larger doses (type II). In at least one of these (C, 115), respiration was also markedly improved; small doses of caffein are evidently capable of restoring the medullary centers when they are

merely depressed rather than paralyzed. In two experiments with low pressure, below 50 mm., the caffeine had practically no effect in either direction.

The blood pressure plateau. Figs. 3 and 4 show that large doses of caffeine eventually lower the blood pressure to a level of between 50 and 65 mm. which pressure is then maintained practically constant, no matter how much additional caffeine may be administered, until death supervenes. In other words, the blood pressure action of caffeine seems to be completed when this level is attained. This phenomenon will be discussed later.

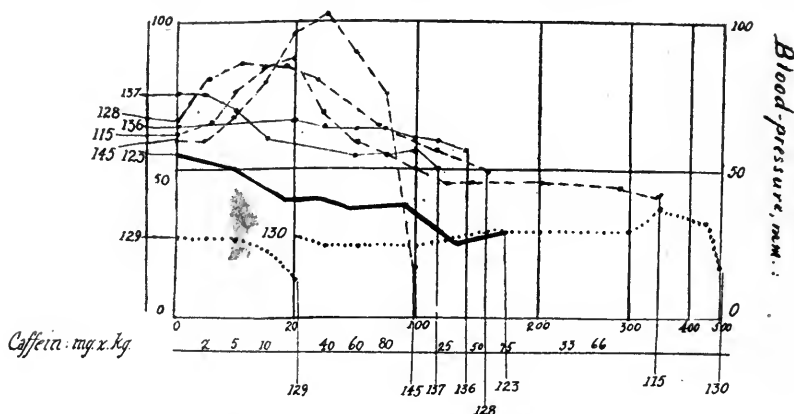


FIG. 5. EFFECTS OF CAFFEINE ON LOW BLOOD PRESSURE

The numbers correspond to the experiments. The curves corresponding to type III are drawn in solid lines (experiment 123, heavy); type II in broken lines, type IV dotted. See text.

Blood pressure with cardioplethysmograms. Since the above was written, one of us (Pilcher) has made a series of fifteen experiments with cardioplethysmographs, involving the opening of the thorax and the use of curare and artificial respiration. The heart was therefore working under unfavorable conditions, and the pressure often falls more rapidly and death occurred with relatively small doses—usually about 140 mg. or below. The general course of the blood pressure is typical; the average curve approaching closely to the line *I b* in fig. 4.

Blood-pressure results of previous investigations. The statements concerning the effects of caffeine on blood pressure, as found

in the literature, appear highly confusing; but a critical examination of the published data shows that this confusion is simply due to the fact that different investigators have centered their attention mainly on one or the other of the phases or doses of caffein. Indeed, their actual results² support the following conclusions which are in satisfactory agreement with the typical effects as we have described them, namely:

Intravenous injection causes an acute and fairly severe *primary fall*. This fall does not occur if the level of blood pressure is low (Swirski, '04). With ordinary blood pressure and ordinary doses, this fall is usually 30 to 40 mm. and is promptly followed by partial, complete, or excessive recovery. If large doses are injected very rapidly, the fall may be greater and proceed to death (Aubert, '72). The fall does not occur on hypodermic administration (Phillips and Bradford, '87; and Reichert, '90, describe a fall after very large doses, hypodermically, but this corresponds to the ordinary late fall). Schroeder, '87, describes fall after intraperitoneal injection of 40 mg.³ per kilo.

Secondary rise. Moderate doses (to 20 or 40 mg. per kg.) usually produce a slight secondary rise 10 to 20 mm. above the original level. This rise may be larger (60 mm.), if the animal struggles (Leven, '68)⁴ or if the vasomotor center has been depressed (Binz, '78)⁵ or if the animal is in poor condition (Vinci, '95).⁶ Large doses—from 40 mg. upward—cause progressive

²Leven, '68; Aubert, '72; Binz, '78; Wagner, '85; von Schroeder, '86 and '87; Phillips and Bradford, '87; Reichert, '90; Vinci, '95; Boek, '00; Gottlieb and Magnus, '01; Santesson, '02; Swirski, '04; Loewi, '05. The references will be found at the end of the last paper.

³The doses in the literature refer sometimes to absolute caffein, sometimes to citrated caffein, etc. We shall always state them in terms of absolute caffein.

⁴Leven, '68; 0.5 gm. per animal, hypodermic; rise of 20 to 60 mm.; i.e., the ordinary rise from moderate doses; rather larger than usual; perhaps from struggling under incomplete anesthesia.

⁵Binz, '78: 100 mg. hypodermic, after alcohol: rise of 40 mm. (ordinary rise, reinforced by passage of narcosis).

⁶Vinci, '95: 10 to 30 mg. per kg., vein and hypodermic: constantly a very slight rise (typical, except the constancy). In animals weakened by repeated hemorrhages (but with blood pressure still fairly high), the caffein rise is much greater; it is also increased, but to a lesser degree, after inanition. This dosage had the optimum effect.

fall of blood pressure (Reichert, '90);⁷ but the pressure maintains itself about 70 mm. till death.

The influence of destruction or paralysis of the vasomotor center has been extensively investigated in the hope of throwing light on the caffein rise. Whenever the blood pressure was very low, it was found that the permanent rise from moderate doses is more uncertain, but a rise *may* occur after section of the medulla (Reichert, '90; Swirski, '04); and also after division of the splanchnic nerves (Phillips and Bradford, '87). This shows that the rise is due, mainly at least, to cardiac stimulation (Swirski attributes it to stimulation of the spinal centers, but as we shall see, the oncometer supports the cardiac explanation).

The more frequent absence of rise after large doses of chloral (Wagner, '85; von Schroeder, '86); the occasional failure in shock; and the lesser rise after arsenic (Pearce, Hill and Eisenbrey, '10), does not prove that the ordinary rise is vasomotor, although it was so interpreted, for instance by Wagner; for it could be explained by injury to the heart, direct or indirect; or by the general interference of low blood pressure with all kinds of circulatory reactions, as will be explained in our second paper.

On the other hand, the occasionally greater rise from a low blood pressure—as observed by Santesson, '02, and in our type II—may be explained by recovery of the vasomotor center, to which caffein may contribute materially.

EFFECT OF CAFFEIN ON THE HEART-RATE

The heart-rate per 10 seconds, was counted by a stop-watch. As the majority of our experiments were made with divided vagi, we shall first discuss these results.

Acute changes in heart-rate. We shall again confine ourselves for the present to the more persistent changes, but will premise that the quickening is temporarily greater immediately after the injection of caffein. Thus, in the average of fourteen injec-

⁷ Reichert, '90: 25 to 60 mg. per kg., jugular vein: typical (primary) fall, recovers or insignificant rise; large doses, marked fall; 35 to 100 mg. per kg., hypodermic: progressive fall.

tions in which the successive changes were recorded, the rate rose from 39 before injection, to $45\frac{1}{2}$ immediately after, to return to $40\frac{1}{2}$ a few minutes later. This maximum rate often coincides with the fall of blood pressure, but this is probably accidental, as the relation between blood pressure and rate is not constant.

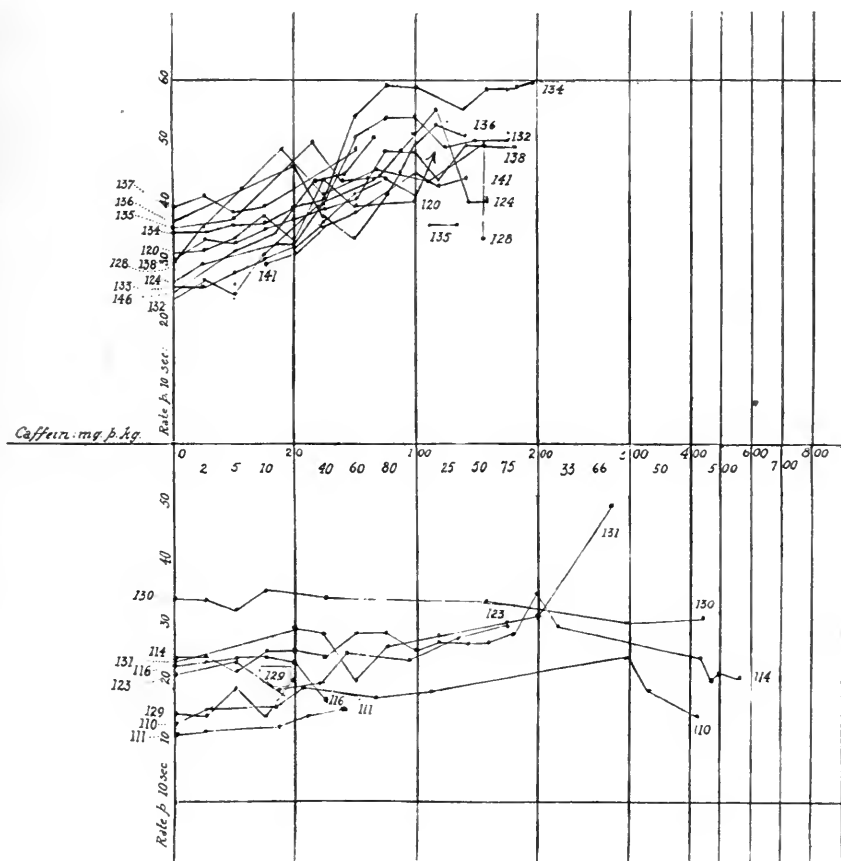


FIG. 6. CURVES OF HEART-RATE UNDER CAFFEIN, VAGI DIVIDED: TYPE I

FIG. 7. CURVES OF HEART-RATE UNDER CAFFEIN, VAGI DIVIDED: TYPE II

Heart-rate with vagi divided. The counts of twenty experiments were charted. The average initial heart rate in these experiments was 27 per 10 seconds, or 162 per minute. The curves can be divided fairly sharply into two groups (figs. 6 to 8). In

type I, which is the larger group, the rate increased rapidly with each successive dose of caffeine. In type II, the increase is much smaller.

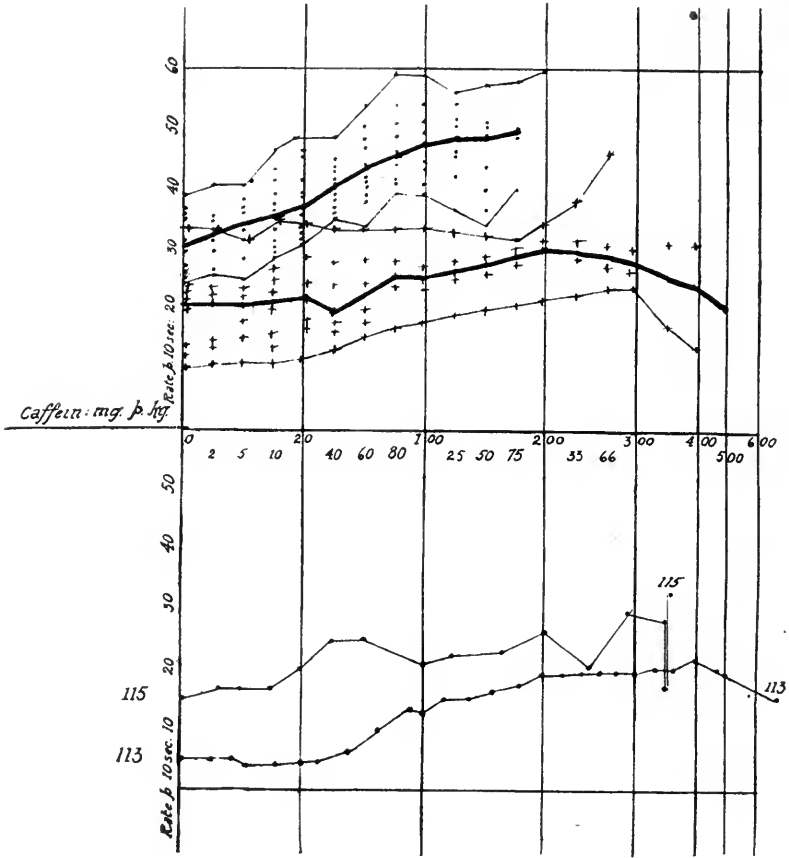


FIG. 8. AVERAGE CURVES OF HEART-RATE, VAGI DIVIDED
FIG. 9. EFFECTS OF CAFFEIN ON HEART-RATE (VAGI INTACT)

The upper curves belong to type I, the lower to type II. The heavy lines are the mean curves, the light lines correspond to the extremes. The individual data are indicated by dots for type I, by crosses for type II.

All the curves of type I are very similar, although the individual curves often show temporary irregularities. The average curve may therefore be taken as representative: Starting with the average initial rate of 30 per 10 seconds, the rate increases rapidly

and progressively with each dose, until about 80 or 100 mg. have been injected. Beyond this, the rate is variable, on account of arrhythmia, but the average rate would remain about the same. The numerical increase and the percentage increase averages as follows for the successive doses:

DOSE OF CAFFEIN	HEART-RATE	NUMERICAL INCREASE	PERCENTAGE INCREASE
<i>mg. per kg.</i>	<i>per 10 sec.</i>		
0	30		
2	32	2	7
5	34	4	13
10	36	6	20
20	37	7	23
60	43	13	43
100	47	17	57
150	48	18	60

The curves of type II run a much less regular course. The most constant feature is the much smaller and more gradual effect, as shown by the following averages:

DOSE OF CAFFEIN	HEART-RATE	NUMERICAL INCREASE	PERCENTAGE INCREASE
0	20		
5	20	0	0
20	22	2	10
60	23	3	15
100	25	5	25
200	30	10	50
300	28	8	40
500	20	0	0

The heart rate in this group does not begin to increase materially until the dosage of 40 mg. is reached. At 100 mg. when type I has reached its maximum rate (57 per cent), type II has increased only 25 per cent, its own maximum (50 per cent) being attained at 200 mg. The difference in the numerical values is even more striking than that in the percentage value.

Factors which influence the response of the heart-rate. Fig. 8 shows very strikingly that the effect is influenced by the initial

heart-rate: In all the curves of type I, the initial rate was above 25, whilst it was below 25 in all but one of type II (and this exceptional experiment—130 should scarcely be counted, since the blood pressure was only 30 mm.). In other words, *cafein causes a much greater quickening (even percentile) in hearts which have naturally a more rapid rate.*

The level of the *blood pressure* has apparently no influence on the phenomena, for high and low blood pressure are fairly evenly distributed between the two types. *Muscular phenomena* also seem to be without influence on the heart-rate (in these experiments with divided vagi), but convulsions often signalize the onset of arrhythmias with excessive tachycardia. It may be remarked that the curves of all animals which had not received curare, belong to type II, but this may be a mere coincidence, since several curare experiments also belong to this type.

The four animals of this series which required extraordinarily large doses of caffeine (over 300 mg.) to produce death, all belonged to type II (see figs. 6 and 7). This indicates that excessive caffeine tachycardia goes hand in hand with cardiac injury; but the number of experiments is too limited to make this conclusion quite secure.

Heart rate with vagi intact. We have only two charted experiments, but as these agree with those of previous investigators, they may serve for illustration (fig. 9):

Small doses may produce a slight primary slowing, if the vagus center is especially excitable (experiment 113). The rate begins to increase when the dosage reaches 10 to 40 mg. The maximum is attained with about 200 mg., when the average rate (23) exceeds the original rate (10) by 13 beats per second or 130 per cent. This indicates escape from vagus control; but that the whole vagus mechanism is still capable of excitation is shown by experiment 115. In this animal after the last dose of 60 mg. of caffeine (total 350 mg. per kg.) and following various reflex irritations, the heart rate fell from 28 to 17 beats. On dividing the vagi, it recovered at once to 33 beats.

Cardiac Irregularities. When the dosage of caffeine exceeds 20 mg., cardiac irregularities of various kinds are very common.

They occurred at some stage in most of our experiments, but as they are usually only temporary, they are not very important in the prolonged course. Our usual experimental methods were not well adapted to their study, but we may record some incidental observations.

The irregularities do not seem to bear any relation to the dosage of caffein. They often appear during or shortly after each injection; they may entirely disappear again with succeeding doses and perhaps reappear later.

In Experiment 114, for instance, (vagi divided) the rate (26 per 10 seconds) remained regular to 140 mg. With the next three injections, each of 20 mg., the rate increased to about 40 and became irregular during and shortly after each injection; but in a few minutes returned to the slower and regular rate. When the dose of 240 mg. was reached, their irregularity became persistent.

These irregularities may concern either the rate or the strength of the beats. Excessively high rates are particularly apt to turn into temporary arrhythmia, especially when the injection is made rapidly or into the jugular vein. Irregularities in the excursions, on the other hand, are most frequently connected with convulsive movements or respiration, or attempts at these under curare.

Significance of the heart-rate for the blood-pressure changes. The cardiac quickening is not an important factor in the blood pressure changes, either for small or large doses, as was pointed out by Swirski, '04, and as may be seen by comparing the average curves of the two phenomena (figs. 4 and 8). Notwithstanding the increased heart-rate, the pressure falls progressively.

Observations of other investigators on the heart-rate. There is substantial agreement regarding this feature of caffein-action. The characteristic effect consists in considerable quickening, increasing with the dose, until the maximum is reached. When the vagi are intact, Swirski, '04, showed that small doses (2 mg. in rabbit, 10 mg. in dogs per kg., intravenous) cause slight slowing in some animals—the majority of rabbits, the minority of dogs. This he showed to be due to stimulation of the vagus

center. This slowing from small doses was recorded by Wagner, '85.

Leven, '63, claimed that large doses of caffein also slow the heart. We have observed this occasionally in our experiments (see figs. 6 and 7) but it is exceptional and usually only temporary. Phillips and Bradford, '87, found the rate increased during the primary fall, and slowed during the secondary rise, but this slowing is presumably only relative, as in our series. Reichert, '90, also observed the quickening during the primary fall.

It is evident that the quickening is not explained by vagus depression, for Johannsen, '69, and all subsequent investigators have shown that it occurs after section of both vagi and even after atropin. However, the vagus tone is lost with large doses (Aubert, '72; Reichert, '90, and our experiment 115), and the response to vagus stimulation becomes uncertain, but the vagus may still cause marked slowing in response to direct or central vagus stimulation, even after doses of 350 mg. per kg.

Nor is the quickening due to accelerator stimulation, for Swirski, '04, found that, when so much caffein had been given, that further doses did not further increase the rate, then accelerator stimulation still gave a further increase. Dixon, '03, found that the quickening occurs after apocodein. It must therefore be attributed to direct muscular stimulation. This was also the conclusion of Cushny and v. Naten, '01.

In the human subject, the response of the heart rate appears to be highly variable, but slowing is perhaps the more usual effect: With 0.1 gm., Wagner records considerable slowing. With 0.3 gm., Leblond reports quickening. With 0.5 gm., Aubert reports slight quickening, Caron slowing. With 1.0 gm., Wilhelm reports slowing. With 1.5 gm., Frehrichs reports quickening. Riegel, with 0.4 to 1.0 gm. hypodermically, reports slowing.

Effects of caffein on the pulse pressure. The excursions of the Huerthle manometer were measured on the tracing and fig. 10 shows these data plotted as curves. The excursions show little change to about 40 mg., whence there is a considerable and progressive increase to about 200 mg. Then the excursions appear temporarily to return toward the normal; but at 600 mg. the average curve has returned to its higher level.

The temporary drop on the curve is very likely accidental, since the number of experiments with these higher doses is small. On the whole, the effect is predominantly that of increased excursions, persisting until death.

These increased excursions might be explained in several ways; as by increased output volume of the heart; by more rapid expulsion of the cardiac contents (so that less blood can flow out of the arteries during the time of contraction) or in the same way, by vasoconstriction; and by lowered arterial pressure (so that a given numerical change will correspond to a larger percentile variation).

Of these possible explanations, the factor of increased rate certainly exists with caffein. Indeed, the average curves of rate (fig. 8) and of excursions (fig. 10) correspond remarkably and this correspondence

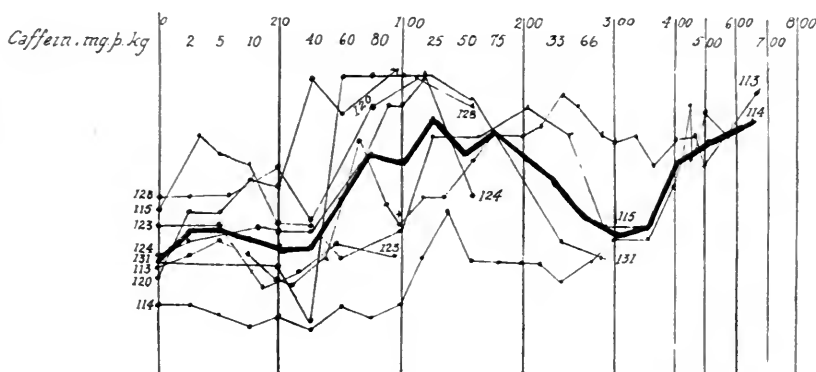


FIG. 10. CAFFEIN ON THE HUERTLE EXCURSIONS

The heavy line represents the mean curve of the plotted experiments.

holds true also of individual experiments (e.g., 113 and 120). It may therefore be safely assumed that the increased excursions are due essentially to the quickened stroke; this makes it impossible to estimate what, if any, part is played by the other theoretical factors.

PERSISTENT EFFECTS ON THE VASOMOTOR CENTER

These were observed by the special method which we have described in a previous paper⁸ (artificial perfusion of an organ,

⁸ T. Sollmann and J. D. Pileher: Amer Journ. Physiol., 1910, vol. 26, p. 233.

with preservation of the central nervous connections). The results are plotted in fig. 11. Since the curves represent the rate of flow through the vessels, a fall of the curve corresponds to central vasoconstriction, and vice versa.

The individual curves run quite irregularly, which in itself show that the *vasomotor center is not greatly concerned in the constant blood-pressure changes*. With 2 mg. doses, the center is often stimulated moderately. This is shown nicely in the tracing of fig. 12. This stimulation is maintained about constant

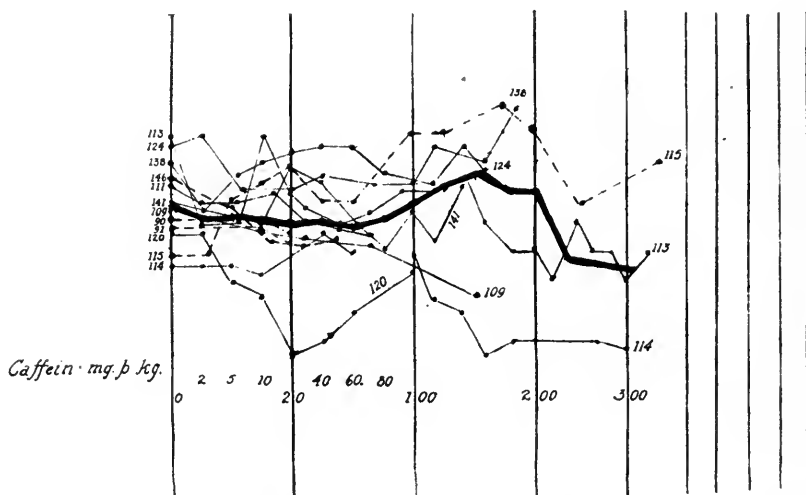


FIG. 11. CAFFEIN ON VASOMOTOR CENTER (RATE OF FLOW OF PREPARED ORGANS)

The broken lines represent the experiments with kidney, the solid line those with spleens. The heavy lines show the mean curve. (Those experiments in which the vasomotor center was known to be injured are excluded.)

till the 60 mg. dose is reached, although the general blood pressure is falling. Between 60 and 150 mg., there is considerable tendency to vasodilation, but with 300 mg. this has returned to a further constriction.

The general tendency, therefore, is toward stimulation of the vasomotor center, even with large doses, and in sharp contrast to the progressive fall of blood pressure.

PERSISTENT EFFECTS ON THE ORGAN-VOLUME

Oncometric observations on the persistent effects were made on ten animals. (The acute changes will be discussed in paper V.) In practically all of these, the curves for the oncometer and blood pressure run parallel, as illustrated by fig. 13. This correspondence indicates that the *early rise and late fall of blood pressure*

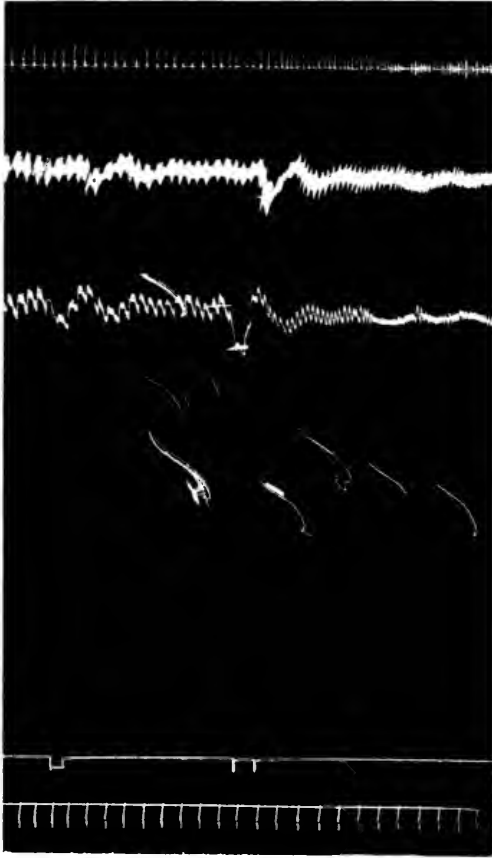


FIG. 12. EFFECT OF CAFFEIN ON RESPIRATION, HUERTHLE AND MEAN BLOOD PRESSURE, AND VASOMOTOR CENTER (OUTFLOW FROM PREPARED SPLEEN)

Injection of caffein, 2 mg. at the first signal, and 5 mg. at the second signal. (Experiment C, 111-1, vagi divided.)

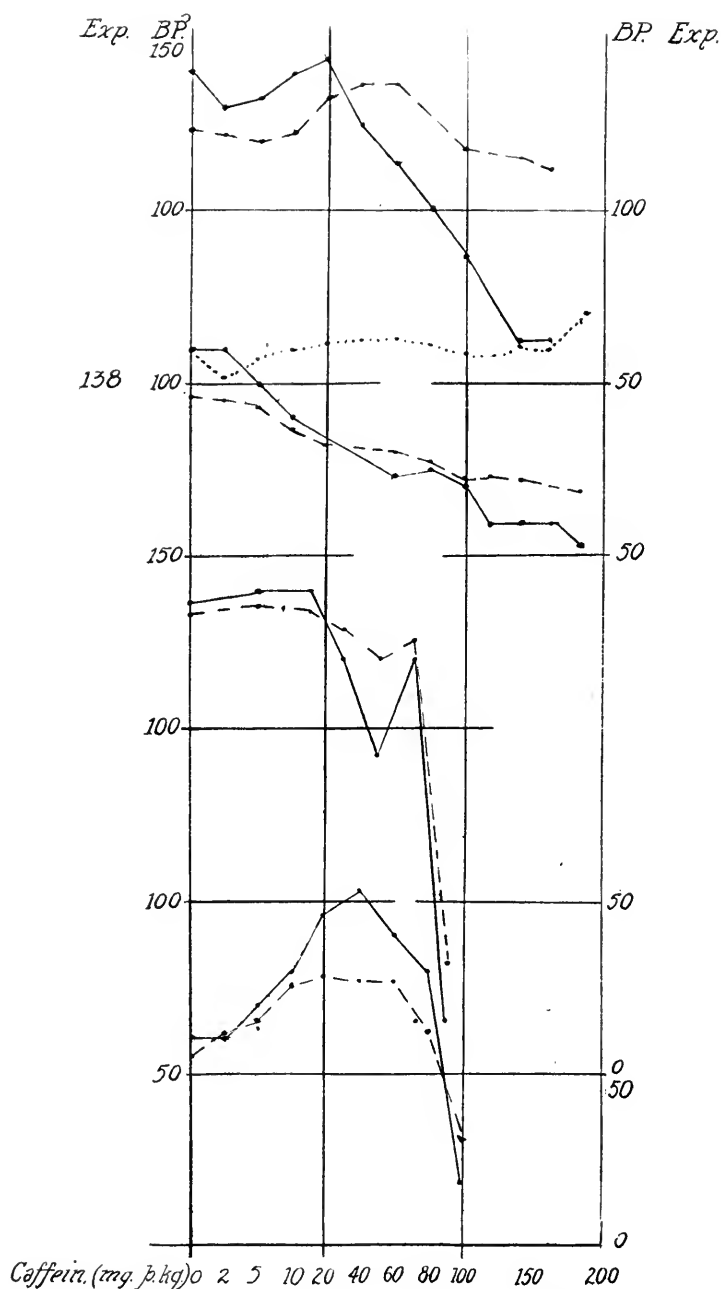


FIG. 13. CURVES OF BLOOD PRESSURE (—) AND ONCOMETER (---)

The vagi were divided in all these experiments; 134 and 138 are from dogs, 145 and 147 from cats. In 134 and 147 the oncometer was applied to the spleen; in 138 and 145 to the kidney. The dotted line in 138 shows the vein flow from the spleen, prepared for the vasomotor center.

are due essentially to cardiac changes. This does not exclude vascular changes as coöperative factors; indeed we shall show that the larger doses of caffein paralyze the blood vessels, and exceptionally there was evidence of effective vaso-constriction in the smaller dosage, *i.e.*, the oncometer fell whilst the blood pressure rose or remained level (in experiment 141, 10 mg., in experiment 146 to 50 mg.).

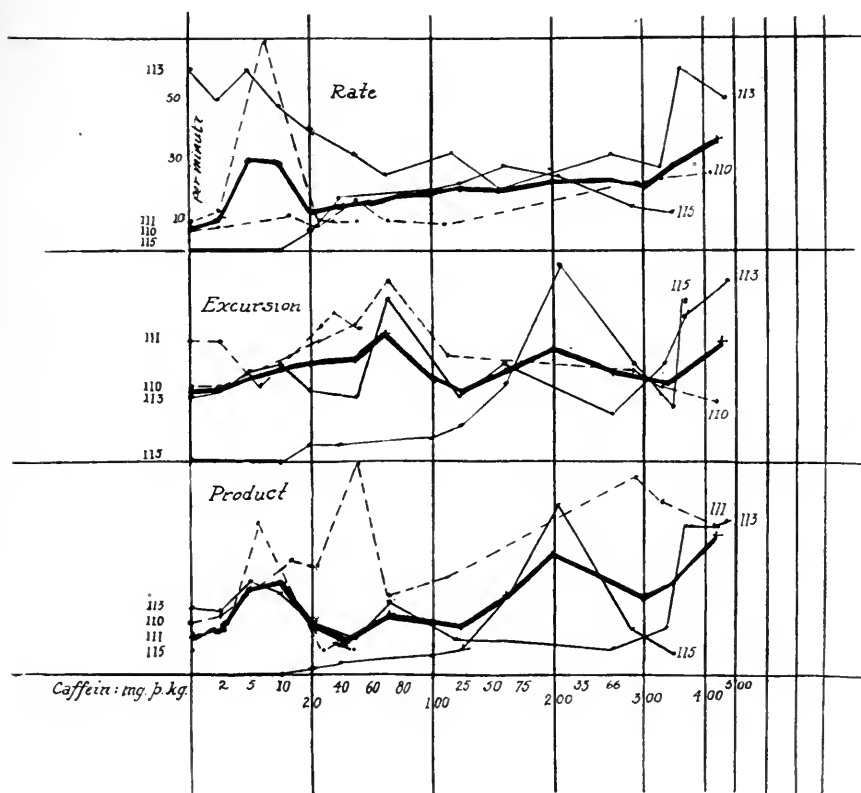


FIG. 14. EFFECTS OF CAFFEIN ON THE RESPIRATION

Recorded by tracheal tambour with open side tube. The upper figure represents the rate, the middle the depth, and the lower the product of rate and depth. The broken lines correspond to experiments 110 and 111, in which the vagi were divided; the solid lines refer to experiments 113 and 115, with vagi intact. The heavy lines show the mean of the four experiments.

EFFECTS ON RESPIRATION

Figure 14 shows the curves of respiration in the four experiments in which it was recorded. The respiration under caffeine is irregular, but in the average it is considerably increased in depth and particularly in rate, or both. The increase is often very considerable (fig. 1 and 12). It is quite pronounced with 5 mg. doses, and persists to 500 mg. or until death; the respiration ceasing only with the final cardiac failure (figs. 15, 16 and 17). Experiment 115 is interesting as illustrating the revival of a spontaneously arrested respiration by caffeine: Artificial respiration had to be maintained until 10 mg. of caffeine had been injected, when spontaneous breathing became reestablished (see fig. 14).

So far as we may judge from our limited number of experiments, the phenomena are essentially similar, whether the vagi are intact or divided.

The stimulation of respiration by caffeine is of course, well known (Leven, '68; Wagner, '85), Binz, '78, found a striking increase, especially in the excursions, from 10 mg. per kg., in heavy alcohol narcosis.

CONVULSIVE PHENOMENA

A record was kept of the convulsive phenomena which occurred frequently in the course of the experiments. These were of several types. With relatively small doses, they resembled struggling rather than convulsions, and we gained the impression that they were caused indirectly, through the partial removal of the narcosis by the central caffeine stimulation, rather than by any direct convulsant action. Somewhat violent convulsions are apt to occur during the abrupt fall of blood pressure which accompanies the intravenous injections; these also may be explained as indirect, due to the sudden anemia of the nervous centers. With larger doses, however, a more persistent convulsant condition appears, the convulsions increasing with the dose; from about 150 mg. per kg., there is usually more or less persistent tetanus of the

strychnin type. The dosage at which the true caffein convulsions appeared is difficult to determine, and appeared to vary greatly, as might be expected in narcotized animals. The possibility of indirect convulsions does not appear to have been recognized by those who have previously described the convulsions, and this may be responsible for some confusion. For instance, Uspensky, '68, and Aubert, '72, claim that the convulsions are prevented by artificial respiration. In our series, the true caffein convulsions occurred about as violently, and with about the same doses, with artificial as with natural respiration. In the case of Aubert at least, there can be little doubt that he was not dealing with the true caffein convulsions, but with the asphyxial convulsions, resulting from the rapid fall of blood pressure, which in turn is contingent on the rapid intravenous injection of massive doses, as practiced by him. The very short duration of his convulsions, even when untreated, scarcely admits of any other explanation.

FATALITY

Whilst the blood pressure, and most other phenomena bear a very constant relation to the total dose, no such constancy is observable in the fatal dose; on the contrary, this is subject to very great variation or idiosyncrasy, under the conditions of our experiments.

The fatalities in the experiments available for this purpose were as follows:

Below 50 mg. per kilo.....	none	160 to 200 mg. per kilo.....	6
57 to 60 mg. per kilo.....	2	300 to 360 mg. per kilo.....	3
70 to 100 per kilo.....	3	450 to 560 mg. per kilo.....	3
130 to 140 mg. per kilo.....	3	800 mg. per kilo.....	1

The series included three cats, one of which died with 90 mg., one with 100 mg., and one required more than 310 mg. per kilo.

The mean fatal dose for all the animals is 175 mg. and nearly half the animals died within the relatively narrow range of 130 and 200 mg.; but some were killed by a dose only one-third as large as the average, whilst others survived doses two, three and even four times as high as the average fatal dose.

We incline to the belief that the latter would have succumbed to the average fatal dose, if this had been given more time to develop its full action. In other words, we believe that there is often a considerable latent period between the injection of the fatal dose, and the actual occurrence of death, corresponding to the period during which the blood pressure continues steadily about the 60 mm. level. In our routine, the injections usually extended over one to three hours; perhaps half or two-thirds of this time was used to reach the dosage of 175 mg.; so that the "latent period"—if this is the correct explanation—may be as much as an hour.

To test the matter, we stopped injections, in some of the experiments, when a presumably fatal dose had been introduced, although the animals were still alive.

The following results were obtained:

Experiment 131.	Total dose, 240 mg. per kilo.	Pause of 30 minutes alive, 360 mg. per kilo.	Died 15 minutes later
Experiment 133.	Total dose, 200 mg. per kilo.		Died 1 hour later
Experiment 136.	Total dose, 140 mg. per kilo.		Died 15 minutes later
Experiment 138.	Total dose, 180 mg. per kilo.		Died 15 minutes later
Experiment 141.	Total dose, 140 mg. per kilo.		Died $\frac{1}{2}$ hour later

These results show that there is in fact a latent period which in these experiments may extend over one-fourth to one hour. This must be an important factor in the variable lethal dose, when repeated injections are made.

When a fairly uniform technic is followed, however, as was the case in most of our experiments, this factor should be uniform, and the apparent lethal dose should therefore also be uniform. It is therefore evident that other factors must also be important.

It impressed us that in many cases the immediate cause of death is not so much the direct action of caffein, but rather a lessened resistance of the heart to unfavorable conditions. The abrupt cardiac stoppage favors this view. As injuries would vary greatly in different experiments, the apparent fatal dose would vary accordingly. This is borne out by our records, for in many cases death supervened on some injurious agency. Amongst these, the acute depressant action of rapid caffein injec-

tion doubtless takes a leading part. Other fatalities followed closely on pneumothorax and aortic compression; epinephrin; traction of the carotid artery, etc.,—procedures which would not ordinarily be fatal in the absence of caffein.

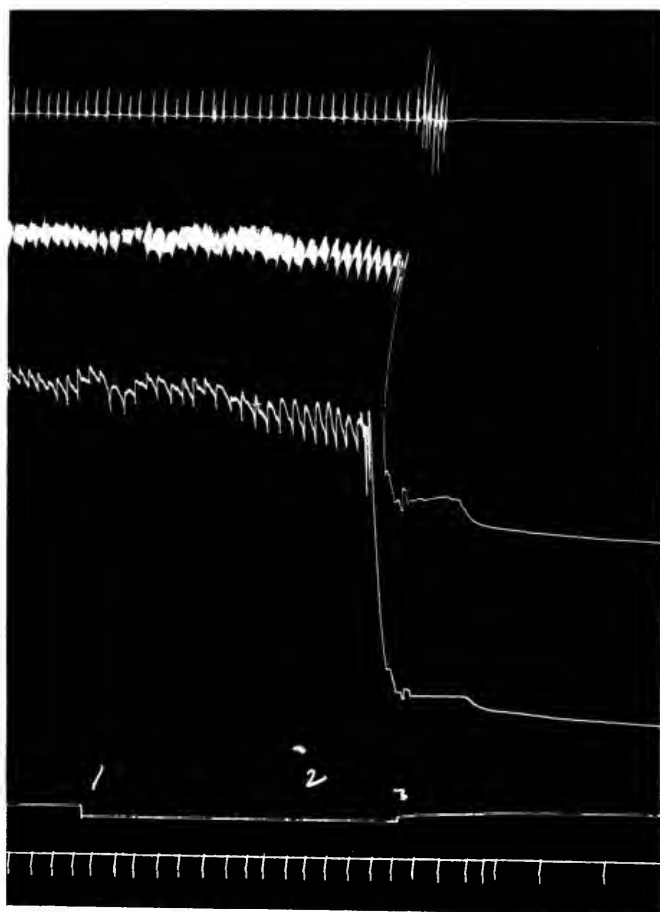


FIG. 15. ACUTE DEATH UNDER SMALL DOSES OF CAFFEIN

The heart stopped acutely during the injection of 20 mg. per kg., the animal having already 37 mg. per kg. *Upper tracing*, respiration; *second tracing*, membrane manometer; *third tracing*, mercurial manometer; abscissa, vein flow from perfused spleen. Experiment C, 111; vagi divided.

The caffein solution entered the vein slowly at 1, rapidly at 2. The cardiac arrest was followed by asphyxial convulsions.

Differences in excretion of caffeine can scarcely be important, since the difference in dosage is much greater than any conceivable difference in excretion. Moreover, the urinary excretion of caffeine must be insignificant when the blood pressure has fallen to the 60 mm. level.

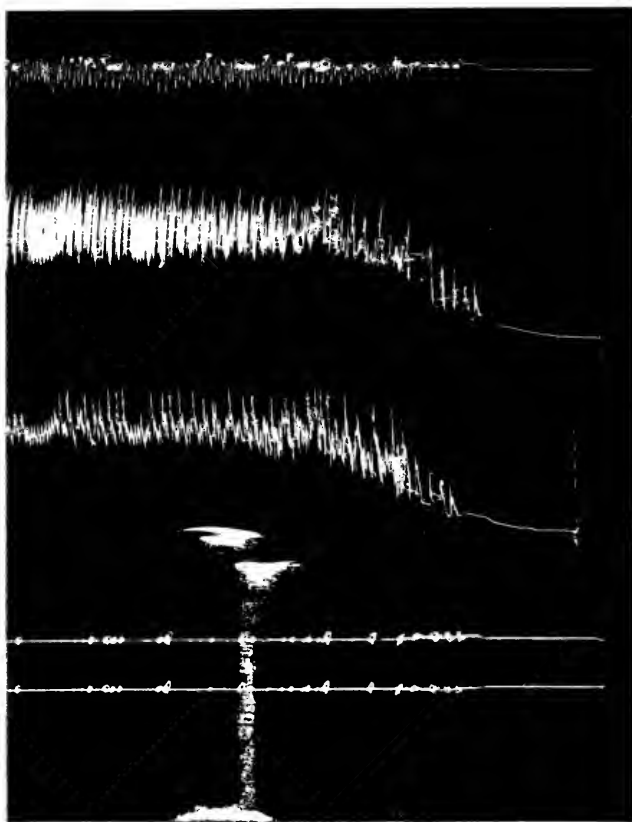


FIG. 16. DEATH AFTER LARGE DOSES OF CAFFEIN

Tracings arranged as in fig. 15. The dots on the abscissa correspond to convulsions. The total dose was 453 mg. per kg. Experiment C, 110; vagi divided.

The immediate mechanism of death, in every case, is cardiac arrest. The respiration may fail almost synchronously; but the blood is usually bright red, and asphyxia can therefore be defi-

nately excluded. The failure of the heart is always rapid. In some cases, and especially with the early fatalities, it occurs instantly, without any warning, usually during an injection (figs. 16 and 17). Ordinarily it is not quite so abrupt, but even here the arrest occurs within one or two minutes after the first

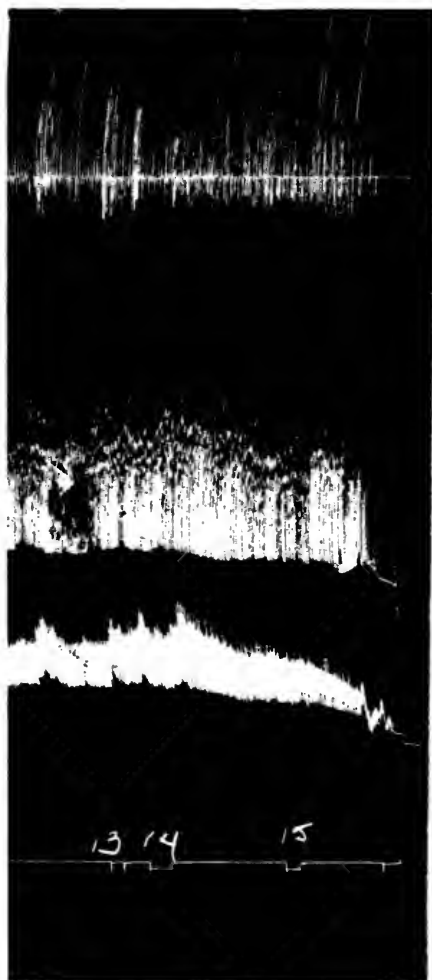


FIG. 17. DEATH AFTER VERY LARGE DOSE OF CAFFEIN (800 MG. PER KG.)

Tracings arranged as in fig. 15. Numbers 13, 14 and 15 represent injections, each of 60 mg. of caffein per kg.

warning, although up to then, the blood pressure may have run level for an hour or more, with repeated injections of caffein.

In those cases in which necropsy was made, the heart was found distended and flabby, twitching feebly and superficially for some time after death.

It is of interest to compare the fatal doses in our experiments with these obtained by Salant and Rieger, '10, with hypodermic and oral administration; but as their preliminary paper does not give details, an exact comparison is not yet possible. They give the following data as to the just fatal dose:

Dogs, 110 mg. per kg., hypodermic.....	symptoms and in some cases fatal
Dogs, 140-150 mg. per kg., mouth....	fatal in a few hours, in some cases
Cats, 150-155 mg. per kg., hypodermic...	symptoms in 10-15 minutes fatal
	in a little over an hour
Rabbits, 160 mg. per kg., earvein.....	fatal
Rabbits, 200 mg. per kg., muscular.....	fatal in few hours
Rabbits, 200 mg. per kg., hypodermic.....	only tremors
Rabbits, 270-280 mg. per kg., hypodermic.....	fatal in 1 to 4 hours
Rabbits, 350-360 mg. per kg., mouth.....	fatal
Guinea pigs.....	similar to rabbits
Pigeons, 140-150 mg. per kg.	somewhat toxic
Frogs, 100 mg. per kg.	toxic

To these may be added the following data from the literature:

Rabbits, 100 mg. per kg., jugular vein, fatal (Upsensky); 250 mg. per kg., hypodermic, just fatal (Falk and Stahlmann). Dog, vein: Aubert found under artificial respiration, that some dogs died with 25 mg. per kg., whilst others survived 300 mg. per kilo. Reichert also records great variability in the fatal dose to dogs, with jugular injections. His fatal doses are remarkably small, ten dogs dying with 26 to 120 mg. per kilo (presumably of citrated caffein, so that the actual doses would only be one half as great). This may be attributed to the great rapidity of his jugular injection (10 to 15 seconds). Death followed very promptly. In only one case was it delayed for eight minutes.

Leven gives the just fatal dose per animal, for hypodermic injection, as 10 mg. for frogs; 150-200 mg. for guinea pigs, 600 mg. for rabbits.

The great variability in the fatal dose makes comparison and definite conclusions difficult. The following, however, appears justified:

The average fatal dose for intravenous and intramuscular injection ranges between 140 and 200 mg. for dogs, cats, and rab-

bits—the differences between species being less pronounced than the differences between individuals, which may vary widely from the average.

This dosage applies, also to hypodermic administration in dogs and cats and to oral administration in dogs.

In rabbits, however, a considerably higher dosage is required by hypodermic and a still higher by oral administration. The range of toxicity appears to apply also to pigeons and frogs.

SUMMARY OF THE DIRECT OBSERVATIONS ON PERSISTENT EFFECTS AND DOSAGE OF CAFFEIN

Before proceeding to the effects of experimental conditions upon the caffein reactions, it will be profitable to restate the essential features of the normal reactions.

1. With doses up to 20 mg., the blood pressure is not much affected, but may show a slight rise; the heart-rate is increased but this bears no relation to the blood pressure. The oncometer usually follows the blood pressure, so that the rise is mainly cardiac; but the vasomotor center is also stimulated, and in some cases the vasoconstrictor factor is more effective than the cardiac factors. A partially depressed vasomotor center may recover under caffein, and the pressure may then rise considerably. The respiration is also stimulated.

2. With doses of 20 to 150 mg., the pressure falls progressively to a constant level of 50 to 70 mm.; the fall thus being large or small according to whether the original pressure was high or low. The heart-rate is further increased, often with irregularities. The central vasomotor stimulation persists. The oncometer follows the blood pressure, so that the fall must be largely cardiac; but the possibility of peripheral vasodilation is not excluded. The respiration is further stimulated.

3. Additional doses produce no further effects until death occurs by rapid cardiac paralysis.

4. The fatal dose for intravenous injection varies in different individuals between 57 and 800 mg. per kilo; but with most animals, it lies between 130 and 200 mg., and 175 mg. may be accepted as a mean.

The cause of this variability has not been demonstrated, but several probable factors are discussed. Amongst these, lessened resistance of the heart to injurious agencies is practically important.

5. Convulsive symptoms may occur from indirect actions as well as from the direct effects of caffein.

II. THE INFLUENCE OF BLOOD-PRESSURE ON CARDIAC AND VASCULAR REACTIONS

INTRODUCTION

The observations of the effect of caffein under ordinary conditions, as described in the preceding paper, led to the conclusion that the heart-rate and the vaso-motor center are practically unimportant in the production of the blood pressure changes, and that these must be mainly of cardiac origin; but that vasodilation by a peripheral action could not be excluded, as a contributing factor.

The theory of exclusive cardiac depression also fails to explain, at least in the light of existing knowledge, why caffein does not lower the blood pressure after destruction of the vasomotor center and why the fall of blood pressure stops at the 50 to 70 mm. level, even when large doses are used.

A priori, one would rather expect that a cardiac depression would progress until death, and that a cardiac fall of blood pressure would therefore also proceed in a smooth curve until death—which is evidently not the case with caffein. The phenomena would be easily explained by vasodilation—for then the pressure would only continue to fall until the vascular paralysis is complete—which might well correspond with the 50 to 70 mm. level. This easy explanation, however, is flatly contradicted by the oncometric observations, which indicate that the fall is mainly cardiac, since the blood pressure and organ volume tend to vary in the same direction.

The condition required further experimental investigation. In the course of this, we soon encountered a fact, the importance

of which does not seem to be generally realized, namely, that *low levels of blood pressure diminish or abolish most if not all blood pressure reaction*. It is, of course, well known that low blood pressure or "shock" abolishes the reactions of the vasomotor center, for instance, to sciatic stimulation. This is currently explained by anemic paralysis of the center. We find, however, that in this condition, the blood pressure also fails to respond to direct stimulation of the efferent vasomotor nerves (splanchnics) and that the absolute rise on the injection of moderate doses of epinephrin is also very much diminished.

Compression of the aorta also gives a much smaller absolute rise; so does clamping of both carotid arteries. Traction of the carotid artery, and intravenous injection of caffein (which produce cardiac depression) also show a much smaller absolute fall when the level of the blood pressure is previously low.

These conclusions are based on the data shown in the Table. (Further details as to method, etc., will be presented in the next paper.)

Influence of Blood-Pressure on Circulatory Reaction

The figures in italics represent the average rise or fall of pressure, in millimeters. The figures in parentheses show the number of animals from which the averages were compiled.

	LEVEL OF BLOOD PRESSURE IN MM.		
	More than 80	50 to 80	Less than 45
Sciatic stimulation (rise).....	<i>32</i> (31)	<i>17½</i> (7)	<i>6½</i> (3)
Splanchnic stimulation (rise).....	<i>45</i> (8)	<i>34</i> (4)	<i>3</i> (5)
Epinephrin $\frac{1}{200}$ mg. per kg. (rise).....	<i>31½</i> (12)	<i>35½</i> (5)	<i>13</i> (1)
Aortic compression (rise).....	<i>65</i> (13)	<i>55</i> (6)	<i>20</i> (2)
Clamping carotids (rise).....	<i>38½</i> (9)	<i>5½</i> (3)	<i>6</i> (1)
Traction carotids (fall).....	<i>28½</i> (8)	<i>22</i> (5)	
Caffein intravenous (fall).....	<i>28</i> (28)	<i>10</i> (13)	<i>2</i> (4)

This table includes experiments with small doses of caffein (up to 40 mg. per kg.); as well as animals which were merely narcotized. As we shall show, these small amounts of caffein do not affect the results materially. Even with these experiments included, the data are not sufficiently extensive to show details,

or to compile an exact curve of the effect of blood pressure on the different reactions, such as has been worked out by Porter⁹ for sciatic stimulation. This deficiency we hope to supply in the future. For the present we have divided the results into three groups, namely, for pressures above 90 mm., pressures of 50 to 80 mm., and pressure of 45 mm. or less. These suffice to show the direction of the change; the averages are so striking, that there can be little doubt that *the circulatory reactions are generally reduced when the level of blood pressure has fallen to 50-80 mm., and that they are almost ineffective when the pressure is 45 mm.*

This interference by low blood pressure cannot be attributed to failure of reaction on the part of the vasomotor center, since most of the reactions to which it applies do not involve this center. It is also known that splanchnic stimulations and especially epinephrin injections, are capable of contracting excised blood vessels; and from this we must assume that the response of the peripheral vasomotor apparatus is not impaired by low blood pressure. Moreover, low blood pressure also lessens the response to aortic compression, in which the arterial system is definitely excluded.

The explanation must therefore be sought outside of the vasomotor apparatus.

One explanation could be, that the heart is so weakened by the low pressure, that it cannot pump effectively against any higher resistance. This is contradicted by the observation that the degree of rise still depends upon the degree of resistance, aortic compression, for instance, causing a greater rise than epinephrin, and this a greater rise than splanchnic stimulation. So far as the cardiac force is concerned, therefore, the pressure could be maintained at a higher level than is actually observed with epinephrin or splanchnic stimulation. Weakening of the heart cannot, therefore, be the essential factor, but it may contribute more or less to the result; large doses of epinephrin ($\frac{1}{2}$ mg. per kg.) raise low blood pressure even more effectively than aortic

⁹ W. T. Porter: Amer. Journ. Physiology, 1907, vol. 20, p. 404.

compression; and there is some evidence that this is due to cardiac stimulation.

If the lessened response of low blood pressure cannot be explained either by vasomotor or cardiac depression, there seems to remain but one other factor, namely, a diminution of the quantity of blood in active circulation in the sense that the quantity of circulating blood is not sufficient to get up a high pressure even when the resistance is raised. Since a large change in the total volume of blood is scarcely conceivable, it would follow that the change must be in the distribution of this blood, namely, a decrease in the proportion on the arterial side of the circulation, and a corresponding increase on the venous or cardiac side—that is, a cardiac dilatation or a venous stasis, a loss of cardiac or venous tone (the latter perhaps in the manner suggested by Y. Henderson¹⁰).

It is evident that the amount of resistance in the arterial system can have but little effect on the pressure, if the quantity of blood in active circulation is not sufficient to distend the arteries. If the blood stagnates mainly in the veins or in the right heart, the state of efficiency of the left heart will be similarly immaterial, for a pump cannot propel more liquid than is supplied to it. A very powerful ventricle would then be no more effective than a relatively weak ventricle.

It is self-evident that these considerations have a most important bearing on the interpretation of "shock," but into this we do not care to enter further at this time.

III. THE INFLUENCE OF CAFFEIN ON BLOOD-PRESSURE REACTIONS

We have shown in the preceding paper that all the blood pressure reactions which we have studied are materially reduced at low levels of blood pressure. When investigating the effects of drugs or other conditions on blood pressure responses, it is therefore necessary to take the level of the blood pressure into account. For this reason, in tabulating our results, and in drawing conclusions, we have considered both, the level of blood pressure, and the dosage of caffein.

¹⁰ Y. Henderson: Amer. Journ. Physiology, 1910, vol. 27, p. 152.

SCIATIC RESPONSE

This was determined by stimulating the cephalic end of the divided sciatic with induction currents more than sufficient to give a maximal blood pressure rise and the average of several such stimulations was noted. The animals were curarized and the vagi divided.

The results may be grouped according to the dosage of caffeine and the level of the blood pressure as follows:

TABLE 1
Blood Pressure response to Sciatic Stimulation

DOSAGE OF CAFFEIN	LEVEL OF BLOOD PRESSURE	AVERAGE RISE	RISE IN INDIVIDUAL ANIMALS*
<i>mg.</i>	<i>mm.</i>		
0	{ 30-45	6	6
	{ 50-80	14	5-8-30
	{ 100+	34	20-20-22-40-45-55
5-20	{ 30-45	6.5	5-8
	{ 50-80	18	15-17-23
	{ 100+	34	12-15-30-45-50
40-100.....	{ 50-80	10	0-5-12-23
	{ 100+	22	15-20-30
140-200.....	{ 30-45	4.5	0-9
	{ 50-80	4	4
300-460.....	{ 30-45	4.5	4.5
	{ 50-80	0	0-0

* These facts are presented graphically in figure 1.

It was shown by Porter¹¹ and confirmed by us¹² that the absolute rise on sciatic stimulation is approximately the same for all blood pressures above about 70 mm.; that it is considerably less between 40 and 70 mm.; and very much less below 40 mm.

¹¹ Porter, H. T.: Amer. Journ. of Physiology, 1907, vol. 20, p. 403.
¹² Sollmann and Pilcher: *ibid.*, 1910, vol. 26, p. 245.

As concerns caffein, fig. 1 shows that doses up to 20 mg. do not affect the sciatic response materially. Larger doses, however, lessen the response conspicuously.

That this lessened response is not due to a paralyzing action on the vasomotor center is shown by our out-flow method, in the following experiments:

Experiment C, 115. Kidney vessels perfused with renal nerves intact. Sciatic stimulation produced the following response:

Caffein dose = 10 mg. per kg. Blood pressure response = $70-100=22$ mm. Outflow slowed.

Caffein dose = 100 mg. Blood pressure response = $50-71=21$ mm. Outflow slowed.

Caffein dose = 350 mg. Blood pressure response = $32-36=4$. Outflow slowed

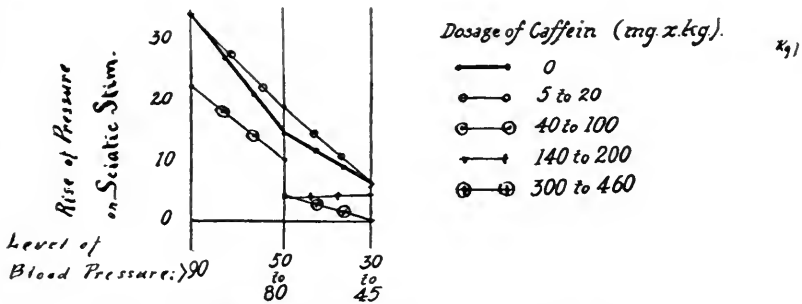


FIG. 1. EFFECT OF CAFFEIN ON SCIATIC STIMULATION

PARTIAL ASPHYXIA

Clamping the tracheal cannula for one minute produced results essentially similar to those of sciatic stimulation, as is shown by the following abstract from Experiment C, 128 (curarized dog):

DOSAGE OF CAFFEIN	EFFECT OF ASPHYXIA ON	
	Blood pressure	Kidney outflow
mg	mm.	units
0.....	70-82 = +12	3.5-3.2 = -0.3
7.....	80-110 = +30	3.6-3.0 = -0.6
117.....	58-65 = + 7	3.6-3.0 = -0.6

It is seen that with the larger dose of caffeine, the blood pressure response is greatly reduced, whilst the response of the vasomotor center is preserved intact.

TETANUS

After large doses of caffeine, the rise of blood pressure during tetanus is very small, but the response of the vasomotor center is preserved (Experiment C, 113, 114 and 115).

II Fig. 2.

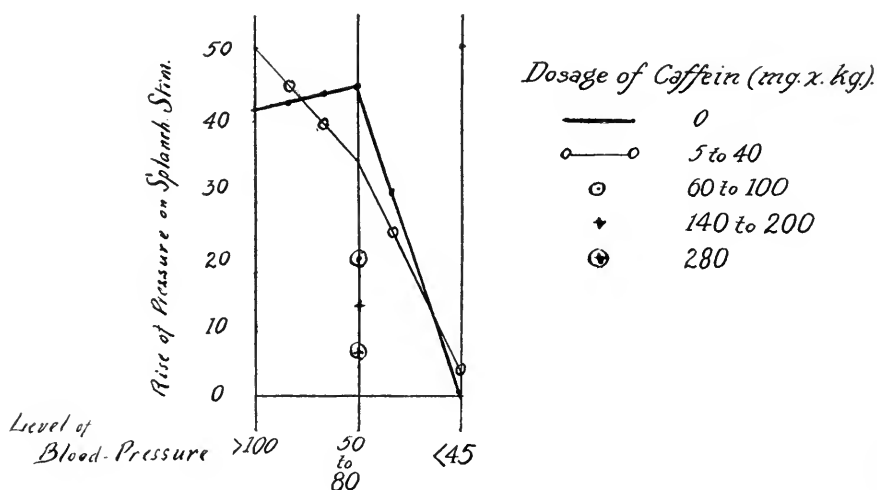


FIG. 2. EFFECT OF CAFFEIN ON SPLANCHNIC STIMULATION

SPLANCHNIC RESPONSE

The splanchnic nerve (usually divided) was stimulated in curarized animals, either just above the diaphragm, or near the left suprarenal gland. The results are shown in table 2 and fig. 2.

TABLE 2

Blood-pressure Response to Splanchnic Stimulation

DOSAGE OF CAFFEIN	LEVEL OF BLOOD PRESSURE	AVERAGE RISE	RISE IN INDIVIDUAL ANIMALS
0.....	<45	0	0
	50-80	45	45
	>100	42½	75.60.39.10.38.33
5-40.....	<45	35	0.0.7.7.
	50-80	34	35.58.18.25
	>100	50½	75.22.95.10
60-100.....	50-80	20	20
140-200.....	50-80	13	23.14.10
280.....	50-80	7	7

In these, the response is not materially affected till the dosage reaches 60 mg.; with larger doses, it is very materially reduced.

RESPONSE TO EPINEPHRIN $\frac{1}{200}$ MG. PER KG. (BOTH VAGI DIVIDED)

This is shown in table 3 and fig. 3.

TABLE 3

Blood-Pressure Response to Epinephrin, $\frac{1}{200}$ mg. per kg.

DOSAGE OF CAFFEIN	LEVEL OF BLOOD PRESSURE	AVERAGE RISE	RISE IN INDIVIDUAL ANIMALS
0.....	50-85	24	15.40.25.17
	>90	34	30.15.25.50.50
5-40.....	<45	13	13
	50-85	42	35.50.28.50.45
	>90	30	10.30.45.40.20.28
75-120.....	<45	12	12
	50-85	28	30.26
	>90	30	25.35
140-200.....	50-85	21	34.20.12.10.20.29.20.22
300.....	<45	6	0.12

Large doses of caffeine again appear to lessen the response, although the difference is not as striking as with sciatic and splanchnic stimulation. It was also noted in a number of experiments that a fair response to epinephrin persisted longer than the response to asphyxia, sciatic or splanchnic stimulation, or carotid traction or occlusion. This indicates that the epinephrin rise,

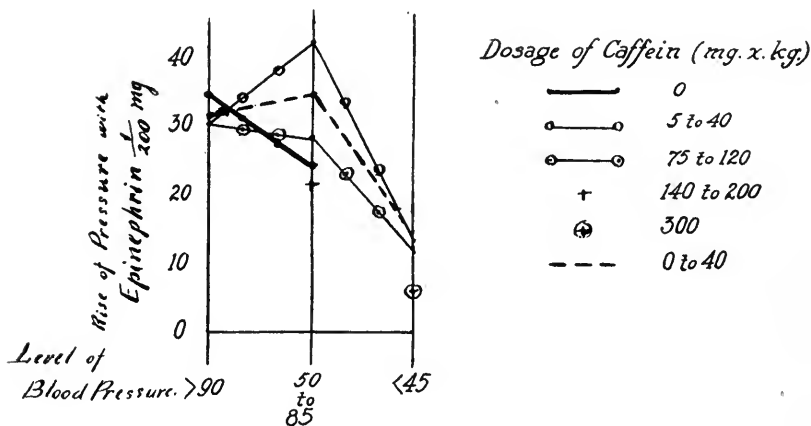


FIG. 3. EFFECT OF CAFFEIN ON EPINEPHRIN, $\frac{1}{200}$ MG.

after large doses of caffeine, may be due to cardiac stimulation, rather than to vaso-constriction, and in fact we have observed that then the oncometer rises with the blood pressure; whereas ordinarily it falls when the blood pressure is raised by epinephrin.

This is shown by the following data:

EXPERIMENT	DOSE OF CAFFEIN	BLOOD-PRESSURE REACTION	ONCOMETER REACTION
135.....	13	115+45	<i>Kidney</i> Fall of 50 units
	93	85+25	Fall of 20 units
	113	80+20	Rise of 10 units
136.....	20	60+45	<i>Spleen</i> Fall of 50 units
	100	60+35	Fall of $1\frac{1}{2}$ units

RESPONSE TO EPINEPHRIN $\frac{1}{20}$ MG. PER KG. (VAGI DIVIDED)

This is shown in table 4 and fig. 4.

TABLE 4.

Blood-pressure response to epinephrin, 1/20 mg. per kg.

DOSAGE OF CAFFEIN	LEVEL OF BLOOD PRESSURE	AVERAGE RISE	RISE IN INDIVIDUAL ANIMALS
0.....	50-85	130	130
	>90	115	135.95
75-120.....	<45	78	83.72
140-200.....	<45	45	69.27
	50-85	61	84.65.35.60.62
>200.....	<45	34	50.18

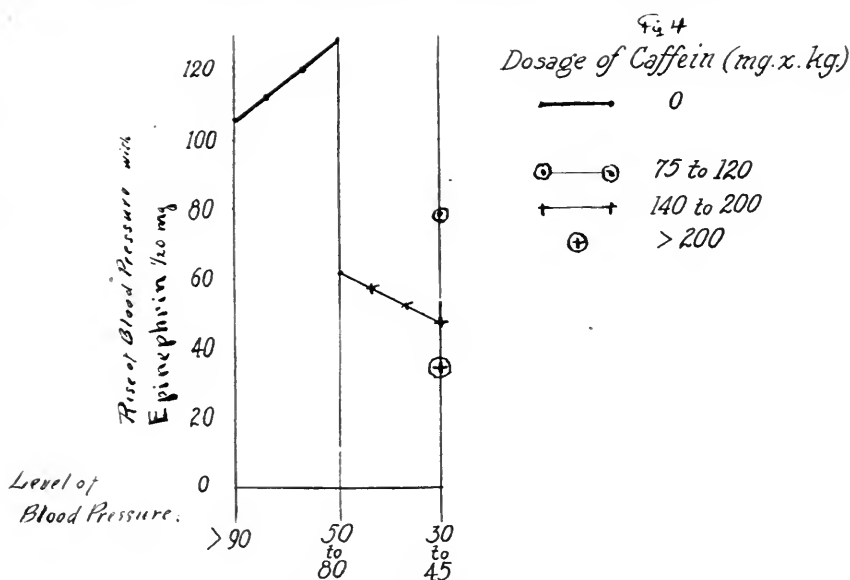


FIG. 4. EFFECT OF CAFFEIN ON EPINEPHRIN, $\frac{1}{20}$ MG.
This figure is one-half the scale of the other curves.

The response is very much greater than with the smaller doses of epinephrin, and the larger dose may produce a moderate or even large rise after the small dose fails:

Experiment 130, caffein, 300 mg.	Response to $\frac{1}{200}$ mg. Epin. = 30+0
	Response to $\frac{1}{20}$ mg. Epin. = 30+15
Experiment 138, caffein, 180 mg.	} Response to $\frac{1}{200}$ mg. Epin. = 55+20
	} Response to $\frac{1}{20}$ mg. Epin. = 58+62

However, as seen in fig. 4, large doses of caffein reduce the response very materially, apparently even more than for the smaller dose of epinephrin. The oncometer again tends to follow the blood pressure, showing that the epinephrin rise after large doses of caffein is cardiac rather than vascular.

It may be remarked, incidentally, that the cardiac stimulation has its limitations. Thus epinephrin has never availed us for reviving a heart which has been arrested by caffein (C, 123 and 132). In two cases, the epinephrin even hastened death. In C, 135 (caffein 133 mg.), the heart failed acutely at the top of the rise (65 to 130 mm.) produced by $\frac{1}{20}$ mg. of epinephrin. In C, 136 (caffein 140 mg.), death occurred soon after the pressure had returned to normal after an injection of $\frac{1}{200}$ mg. of epinephrin. This, of course, could be a coincidence.

AORTIC COMPRESSION

The aorta was compressed against the vertebral column by the finger introduced through a small cut in the thoracic wall. The compression was maintained until there was no further rise in the blood pressure.

The results are shown in table 5.

The results with caffein are not sufficiently numerous to be reliable. So far as they go, they indicate that doses up to 160 mg. do not impair the response (considering the level of blood pressure); but the large dose of 560 mg. gave very much less than the average rise for that level of blood pressure (55 mm.).

Acute cardiac failure was of common occurrence shortly after the compression:

TABLE 5

Blood-pressure Response to Aortic Compression

In Experiment C, 114, the dog had received 560 mg. of caffein per kg. The blood pressure stood at 55 mm. The aorta was occluded twice in rapid succession, each time for 20 seconds, the pressure rising to 70 and 80 mm. The heart failed 3 minutes later.

In C, 115, 350 mg. of caffein, pressure had fallen to 30 mm., heart weak. Aortic compression of 25 seconds raises pressure to 55 mm. The heart continues to fail and stops 2 minutes later.

This, however, was not constant, and it is doubtful whether importance can be attached to it.

DOSAGE OF CAFFEIN	LEVEL OF BLOOD PRESSURE	AVERAGE RISE	RISE IN INDIVIDUAL ANIMALS
0.....	$\left\{ \begin{array}{l} <45 \\ 50-80 \\ >90 \end{array} \right.$	$\begin{array}{l} 20 \\ 77\frac{1}{2} \\ 65\frac{1}{2} \end{array}$	$\begin{array}{l} 70.85 \\ 65.80.45.60.78.80.105.30. \\ 70.33.100.40 \end{array}$
5 to 40.....	$\left\{ \begin{array}{l} <45 \\ 50-80 \\ >90 \end{array} \right.$	$\begin{array}{l} 20 \\ 44\frac{1}{2} \\ 55 \end{array}$	$\begin{array}{l} 20 \\ 48.60.40.30 \\ 55 \end{array}$
60 to 160.....	$\left\{ \begin{array}{l} <45 \\ 50-80 \end{array} \right.$	$\begin{array}{l} 19 \\ 75 \end{array}$	$\begin{array}{l} 50.8.0 \\ 95.55 \end{array}$
350 to 560.....	$\left\{ \begin{array}{l} <45 \\ 50-80 \end{array} \right.$	$\begin{array}{l} 25 \\ 25 \end{array}$	$\begin{array}{l} 25 \\ 25 \end{array}$

COMPRESSION OF BOTH CAROTID ARTERIES

One carotid artery being ligated for the insertion of the manometer cannula, we noted the effect of temporarily compressing the other (right) carotid—usually for one minute. As is well known, the occlusion of both carotid arteries causes a persistent rise of blood pressure.

This is generally attributed to anemic stimulation of the vasomotor center. We rather question this explanation. With the use of our perfusion method, the results are variable and slight, indicating that

the central stimulation cannot be very powerful. On the other hand, we have found that the oncometric volume of the kidney, spleen and intestine is generally increased as the pressure rise, although occasionally there is evidence of constriction. The excursions of the Huerthle manometer also tend to increase as the pressure rises. These data indicate that the central vasomotor stimulation is uncertain and relatively weak, and that the main element in the rise is cardiac.

The effect of caffeine on this rise is shown in table 6:

TABLE 6
Blood-pressure Response to Carotid Compression

DOSAGE OF CAFFEIN	LEVEL OF BLOOD PRESSURE	AVERAGE RISE	RISE IN INDIVIDUAL ANIMALS
0.....	$\left\{ \begin{array}{l} <45 \\ 50-80 \\ >90 \end{array} \right.$	$\begin{array}{c} 6 \\ 6\frac{1}{2} \\ 37 \end{array}$	$\begin{array}{c} 6 \\ 5.8 \\ 20.50.25.40.25.60 \end{array}$
5-20.....	$\left\{ \begin{array}{l} 50-80 \\ >90 \end{array} \right.$	$\begin{array}{c} 4 \\ 42 \end{array}$	$\begin{array}{c} 4 \\ 75.20.30 \end{array}$
40-100.....	$\left\{ \begin{array}{l} 50-80 \\ >90 \end{array} \right.$	$\begin{array}{c} 3\frac{1}{2} \\ 23\frac{1}{2} \end{array}$	$\begin{array}{c} 3.4 \\ 12.22.30.30 \end{array}$
140-200.....	$\left\{ \begin{array}{l} 50-80 \\ >90 \end{array} \right.$	$\begin{array}{c} 7 \\ 5 \end{array}$	$\begin{array}{c} 7.5.10 \\ 5 \end{array}$
>300.....	<45	3	3

Here again it is fairly clear that the smaller doses of caffeine do not influence the reaction; but doses above 40 mg. per kg. plainly give less reaction.

CAROTID TRACTION REFLEX

Sollmann and Brown¹² showed that traction on the cephalic end of the carotid artery, and thereby on the carotid plexus, sets off a reflex which lowers the blood pressure by depressing the heart. The path of this reflex has not been traced. Since it is

¹²Sollmann and Brown: Proc. Soc. Exp. Biol. and Med., vol. 5, p. 20, 1908.

prevented by nicotin, it must pass through a sympathetic synapsis, but it does not go through any of the known cardiac nerves. The fall of blood pressure is accompanied by a fall of the oncometric organ-volume and by diminished excursion of the membrane manometer. The vasomotor center is slightly stimulated, for the perfusion-flow is generally slowed. (The traction was applied during closure of both carotid arteries).

The effects of caffein on this reaction are shown in table 7.

TABLE 7
Blood-pressure Response to Carotid Traction

DOSAGE OF CAFFEIN	LEVEL OF BLOOD PRESSURE	AVERAGE FALL	FALL IN INDIVIDUAL ANIMALS
0.....	{ 50-80 >90	15 28	20.12.14 40.20.30.40.12
5-20.....	{ 50-80 >90	32 28	13.50 65.20.0
40-100.....	{ 50-80 >90	19 17	18.8.6.15.50 22.25.3
140-200.....	{ 50-80 >90	4 $\frac{2}{3}$ 16 $\frac{1}{2}$	4.5.5 15.18
350.....	50-80	12	12

Here once more small doses of caffein have no positive effect, whilst doses above 40 mg. lessen the reaction very materially.

We also attempted to investigate the reaction to *cerebral compression* (dural injections of saline solution under pressure); but the proceeding seemed to cause so much and so uncertain damage in the two experiments (C, 120 and 127), that it was judged unsuitable for comparative observations.

INTRAVENOUS INJECTION OF CAFFEIN

When caffein is injected rapidly into a vein, it causes an acute, but short fall of blood pressure, which is due to cardiac weakening, as we shall show in another paper. The influence of the previous

doses of caffein on this fall is shown in table 8. (From 10 to 20 mg. of caffein per kg. were used for producing the reaction.)

TABLE 8
Blood-pressure Fall Intravenous Caffein Injection

DOSAGE OF CAFFEIN	LEVEL OF BLOOD PRESSURE	AVERAGE FALL	NUMBER OF ANIMALS
5 to 20.....	<45	2	4
	50-80	10	13
	>90	28	28
40 to 100.....	<45	4	3
	50-80	11½	20
	>90	33	24
140 to 200.....	<45	6	5
	50-80	7	14
	>90	16	5
>300.....	<45	0	3
	50-80	2	8

Here, doses of caffein up to 100 mg. do not affect the reaction, larger doses diminish and eventually abolish the fall.

SUMMARY AND DISCUSSION

The blood pressure response to circulatory reactions—cardiac as well as central and peripheral vasomotor—is materially reduced when the level of the blood pressure has fallen to 50 to 80 mm.; and below 45 mm. these reactions are almost ineffective.

This must be taken into account in studying the specific influence of drugs or other conditions on such reactions.

Caffein, above a certain dosage, reduced these reactions; a part of this reduction is due to fall of blood pressure; but the reduction with caffein is markedly greater than obtains for the same level of pressure without caffein. With most of the reactions, the specific reduction becomes apparent when the dosage of caffein exceeds 40 mg. per kilo; with other reactions a

somewhat larger dosage appears to be required—60 to 140 mg. per kg. Doses below 20 mg. do not reduce the reactions. In fact they rather appear to increase them, but these differences are not sufficiently large and constant to be conclusive.

The consideration of the nature of these reactions throws some light on the mechanism of these specific actions of caffein:

The lessened response to sciatic stimulation, asphyxia and tetanus indicate that caffein depresses the vasomotor system. This depression is not central, for our perfusion method shows that the vasomotor center continues active even with very large doses of caffein. The depression must therefore be peripheral. This is confirmed by the relative inefficiency of splanchnic stimulation and of epinephrin.

Caffein in large doses therefore depresses the peripheral vasomotor apparatus. In addition, however, caffein interferes also with reactions, which are largely or purely cardiac. The response to aortic compression does not become reduced until a high dosage of caffein has been reached; the heart also continues to respond in a considerable degree to epinephrin; but the response is reduced. With carotid compression, the reduction is very pronounced.

Caffein in large doses therefore reduces the efficiency of the heart. It will be shown in a later paper that this lessened efficiency consists mainly in cardiac dilation, i.e., in lessened tone of the cardiac muscle.

There is also marked interference with those cardiac reactions which result in a fall of blood pressure; namely, carotid traction and intravenous injection of caffein. This is presumably also connected with the cardiac depressant effect of large doses of caffein, but we are not prepared to furnish a detailed explanation.

IV. THE EFFECTS OF CAFFEIN ON THE VESSELS OF EXCISED ORGANS. A METHOD OF DEMONSTRATING VASODILATOR ACTIONS

In the preceding section, we adduced evidence that caffein must have a peripheral depressant action on blood-vessels; adequate doses, for instance, diminish the response to splanchnic stimulation and to epinephrin. We attempted to demonstrate

this directly by post mortem perfusion of dogs' kidneys with warmed normal saline solution; measuring the outflow and the kidney volume, first with the pure solution, then with the addition of various proportions of caffein. We found, however, that caffein concentrations from 1:10,000 up to 1:100 produced very little effect.

It occurred to us that this failure might be due to the fact that the vessels are already partly dilated after death; and that caffein whilst it did not produce an active dilation, might still prevent constrictor effects. We therefore tried whether the constrictor response to epinephrin perfusion could be influenced by caffein; by comparing the effects of perfusion with epinephrin solution, with those of mixtures of epinephrin and caffein. As will be seen in the tabulation at the end of this paper, this was indeed the case: Even dilutions of 1:10,000 reduces the epinephrin constriction to a remarkable degree; 1:1000 gave a still greater reduction—the absolute result depending on the relative proportion of the two agents.

We have not tried this experiment on other organs, but there is no reason to anticipate that the results would be different; nor have we tested caffein against other constricting agents, but the analogous effects of caffein on all the constrictor reactions in the intact animals would lead us to expect a similar behavior. It is scarcely necessary to point out that this method of simultaneous perfusion with antagonistic drugs may have other valuable applications.

Quite a number of observations on caffein-perfusion of excised kidneys are on record. These show a general tendency to dilation, but very often there was no result. The difference can probably be explained by the presence or absence of a pre-existing vasoconstriction:

Kobert, '86: Found 1:1000 negative; 1.2:1000 gave fair dialation.

Munk, '87: Slight increase of flow.

Sakusoff, '04: 1:1000, increase.

Beco and Plumier, '06: Caffein, theophyllin and theobromin caused marked increase in kidney, less powerful in leg; with caffein, the dilation appeared to be preceded by constriction.

Sollmann and Hatcher, '08: 1:20,000 to 1:10,000 no increase in sixteen experiments, fair increase in one.

CONCLUSIONS

This furnishes definite proof that caffein produces a peripheral vasomotor-depression; but this action appears to differ from that of chloral and hydrocyanic acid; for these drugs dilate the renal vessels directly, whilst the caffein acts only by preventing constriction.

The concentrations of caffein which proved effective for this purpose indicate that ordinary therapeutic doses must have this effect, for a concentration of 1:10,000 would correspond to a dose of 0.1 mg. per kilo, if this were evenly distributed through all the tissues, and this would correspond to 7 mg. or $\frac{1}{10}$ grain for a 70 kg. patient.

Tabulation of Post-Mortem Perfusion Experiments. The Changes Are Stated in Terms of Arbitrary Units

EXPERIMENT C, 140	CHANGE IN	
	Oncometer	Flow
Caffein, 1:1000.....	+0.2	+0.5
Caffein, 1:100.....	+0.12	+3.65
Epinephrin, 1:100,000.....	-11.3	Completely arrested
Epinephrin, 1:100,000+caffein 1:100.....	-5.9	Not completely arrested
Difference with caffein.....	+5.4	+0.8
Epinephrin, 1:500,000.....	-0.3	-1.4
Epinephrin, 1:500,000+caffein 1:100.....	-0.4	-0.9
Difference with caffein.....	-0.1	+0.5
Epinephrin, 1:333,000.....	+0.4	-1.0
Epinephrin, 1:333,000+caffein 1:1000.....	+0.5	+2.2
Difference with caffein.....	+0.1	+3.2
Epinephrin, 1:100,000.....	-1.0	Completely arrested
Epinephrin, 1:100,000+caffein 1:1000.....	-0.5	Not completely arrested
Difference with caffein.....	+0.5	+2.2

EXPERIMENT C, 141	CHANGE IN	
	Oncometer	Flow
Caffein, 1:10,000.....	+0.1	0
Caffein 1:1000.....	+0.3	+0.8
Epinephrin 1:333,000.....	-7.0	Arrested
Epinephrin 1:333,000+cafein 1:10,000.....	-4.5	Not completely arrested
Epinephrin 1:333,000+cafein 1:1000.....	-1.2	Not completely arrested
Difference with caffein, 1:10,000.....	+2.5	+0.6
Difference with caffein, 1:1000.....	+5.8	+3.8

V. ACUTE EFFECTS OF INTRAVENOUS CAFFEIN INJECTIONS ON THE CIRCULATION

CONTENTS

Primary rise.....	68
Acute fall.....	69
Later changes.....	76
Relative effect on different splanchnic organs.....	83
Principal types of blood-pressure and oncometer changes.....	86
Summary.....	88
Practical applications of the circulatory action of caffein.....	89
Conclusions.....	91
Bibliography.....	92

INTRODUCTION

When caffein is injected into a vein, the typical, persistent effects which were described in the first paper, are complicated by the acute actions of the concentrated caffein solution on the circulatory apparatus. The results are fairly uniform in their main features, although there are quantitative differences which render the total picture quite complex. In analyzing these, we have found it convenient to divide the phenomena into periods, corresponding to changes in the blood pressure, as illustrated in fig. 1:

At the beginning of the injection, the blood pressure rises slightly by 5 or 10 mm. (period I=5 seconds). Then it falls immediately by about 30 mm. (period II=15 seconds). Within a few seconds after the completion of the injection, it begins to recover rapidly. This acute rise (period III) is completed in about half a minute. The level reached may be the original, or slightly above, or more or less below. The further changes (period IV, 2 minutes; period V, 4 minutes) are variable, but not

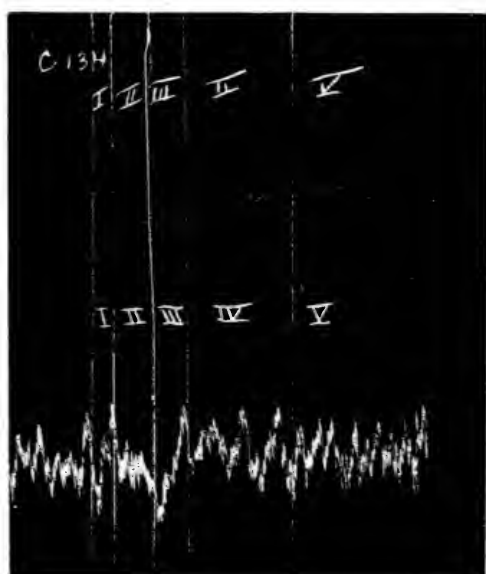


FIG. 1. EFFECTS OF CAFFEIN ON SPLEEN-VOLUME (UPPER TRACING), AND MEAN BLOOD PRESSURE (EXPERIMENT C, 134)

Ten milligrams of caffeine after a preceding injection of 10 mg. (The Roman numerals indicate the successive stages of the action, as explained in Paper V).

very extensive. Within five or ten minutes after the completion of the injection, the blood pressure has usually reached a fairly constant level.

PRIMARY RISE—THE FIRST PERIOD

As the injection enters the vein, the blood pressure shows a small abrupt rise of 5 to 10 mm. (fig. 2). This is accompanied by a corresponding elevation of the oncometer, so that it must be essentially cardiac. There may be some stimulation of the vasomotor

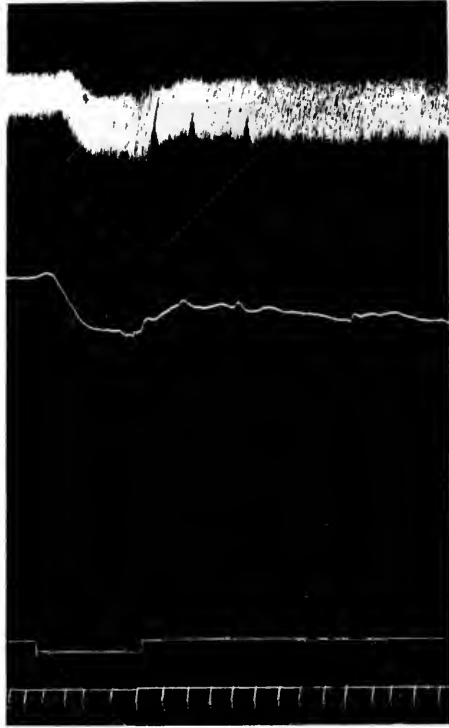


FIG. 2. CAFFEIN 40 MG. PER KG., INTRAVENOUS, EXPERIMENT C, 109-4
VAGI INTACT

Dog had previously received 30 mg. *Upper tracing*, membrane manometer; *second tracing*, mercury manometer; *lowest tracing*, flow from perfused spleen.

center as shown by diminished perfusion flow; but the oncometer shows that the cardiac element predominates. (Experiments C, 136 and 137.)

This primary rise is not characteristic of caffeine, but may be referred to the acute influx of fluid.

ACUTE FALL—THE SECOND PERIOD

This is characterized by an acute fall of blood pressure, which is invariably accompanied by a synchronous and fairly proportional fall of the oncometer (figs. 3 and 4). This correspondence is



FIG. 3. CAFFEIN (10 MG.) ON ONCOMETER (MIDDLE TRACING) AND BLOOD PRESSURE (LOWER TRACING)

The kidney oncometer tracing is plotted from the readings. It is of the type "A2" (Experiment C, 145). The cat had received 10 mg. of caffein.

seen whether the oncometer is attached to the kidney, spleen or intestine, in dogs or in cats. *The blood pressure fall is therefore essentially cardiac.*

The vasomotor center indeed usually gives some evidence of stimulation (i.e., slowing of the perfusion flow; see fig. 2); this



FIG. 4. CAFFEIN 20 MG. ON KIDNEY ONCOMETER (UPPER TRACING) AND BLOOD PRESSURE (SECOND TRACING)

The oncometer tracing is of the type "K2." The dog (C, 135-5) had received 115 mg. of caffein; the heart is very arhythmic.

may be indirect, due to the central anemia from the fall of blood pressure. The respiratory center is similarly stimulated (fig. 5).

The simultaneous fall of blood pressure and oncometer has been described by the previous investigators (Philips and Bradford, '87, Gottlieb and Magnus, '01; Loewi, Fletcher and Henderson, '05). Roy and Sherrington, '87, observed a simultaneous decrease of the brain volume.

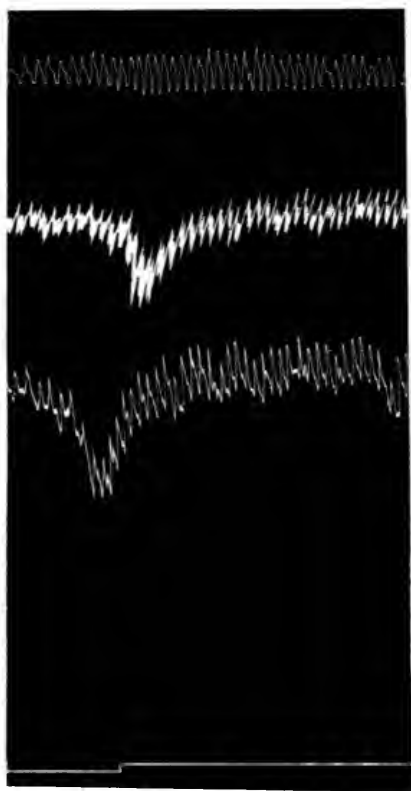


FIG. 5. EFFECTS OF CAFFEIN ON RESPIRATION AND BLOOD PRESSURE (EXPERIMENT C, 110-1)

Injection of 16.5 mg. of caffein per kg. (vagi divided). *Upper tracing*, respiration from tambour connected with trachea, with open side-tube. *Middle tracing*, carotid pressure tracing by Harvard membrane manometer. *Lower tracing* mean pressure by damped mercury manometer. The *signal line* in all the figures represents zero pressure of the mercurial manometer.

All the tracings are reduced to about $\frac{2}{3}$ natural size. They read from left to right, the drum moving about 17 mm. per minute.

Phillips and Bradford considered the fall of blood pressure as cardiac; but they believe that the fall of the oncometer is not due to the fall of blood pressure. Their evidence for this conclusion is rather unsatisfactory. They base it mainly on failure of the two curves to correspond precisely. It seems to us, however, that the kidney volume could scarcely be expected to follow the blood pressure instantly and exactly, since other vascular areas are also involved. As a matter of fact, their experiments show a reasonable correspondence of the two curves, save in the rare cases in which the blood pressure does not fall. In these one may assume that the central vaso-constrictor factor is unusually pronounced. However, such a strong and universal vasoconstriction as they assume in explanation of the fall of oncometer, seems quite incompatible with a fall of blood pressure, unless the heart were enormously weakened, much more so than corresponds to the actual observations.

They found, further, that the fall of blood pressure and oncometer occurred after section of the splanchnics, so that the vaso-constriction which they assumed would be due to a peripheral action on the vessels. Our perfusion experiments refute this assumption. In fact, the persistence of the actions after section of the splanchnics speaks very strongly for their cardiac origin. The contraction of the brain-volume also conforms better to the cardiac explanation.

Further evidence that the acute fall is mainly cardiac, is furnished by the fact that it may occur when the vasomotor center has been destroyed—although the low blood pressure generally interferes with a typical fall.

The cardiac inefficiency is not due to altered heart-rate, for the rate is actually increased during the fall (for instance, in experiment C, 124, with the second injection of 10 mg. of caffein, the rate before injection was 31 beats per ten seconds; during the fall it rose to 40 beats; afterwards it increased further to 45 beats).

An attempt was made to obtain an explanation of the mechanism of the fall by measuring the systolic and diastolic excursions of the membrane manometer. The results are shown in the following table.

With normal blood pressure, therefore, the systolic pressure falls less than the diastolic pressure, so that excursions appear increased (see figs. 2 and 5); with low blood pressure, the systolic falls as much or more than the diastolic pressure, so that the excursions appear dimir-

THE DIASTOLIC PRESSURE FALLS	NUMBER OF INJECTIONS WITH BLOOD-PRESSURE	
	Over 100 mm.	Below 100 mm.
More than the systolic, resulting in greater excursions.....	20	1
Same as the systolic, resulting in same excursions.....	3	8
Less than the systolic, resulting in smaller excursions	4	9

ished. (Phillips and Bradford also found the excursion of the spring manometer lessened.)

A decrease in the cardiac output—which is proven by the fall of the oncometer—would be supposed to lessen the systolic pressure at least as much as the diastolic. Consequently, there must be some other factor to account for the smaller fall in systolic pressure. Two such factors occur to us:

a Altered relative duration of systole and diastole: The shorter the systole, the less blood could escape from the aorta into the peripheral vessels during the time of contraction. Inversely the longer the diastole, the more will the diastolic pressure fall.

b Vasodilation: The reduction of aortic pressure by escape of blood into the peripheral vessels must be more pronounced in the naturally longer diastolic period, and this would be exaggerated by any vasodilation.

We have not investigated whether there is actually a shortening of the systole, relative to the diastole, but we have shown that caffein is capable of producing pronounced vasodilation. This factor is also favored by the fact that, when the blood pressure is low, the systolic pressure falls as much or more than the diastolic pressure, for when the blood pressure is low, the vessels are already dilated, the vasodilator action would therefore not be as effective, and hence the fall of diastolic pressure could not be so conspicuous.

It would appear, therefore, that there is a vasodilator factor in the second period, although this is overshadowed by the cardiac weakening. The direct proof of the latter will be presented by one of us (Pilcher) in a later paper.

The influence of various factors on the caffein fall. We have already discussed the influence of low blood pressure (see part II) and the

influence of preceding doses of caffeine (part III). Low blood pressure interferes with the caffeine fall just as it interferes with other circulatory reactions, presumably by altering the mass of actively circulating blood. Large doses of caffeine interfere with the fall; indirectly through lowering the level of blood pressure, and also directly, presumably by the permanent cardiac depression.

We still have to consider certain other factors: The effect increases, naturally, with the *concentration of the caffeine* in the blood, and therefore with the quantity introduced with each dose (see fig. 12 of first paper), until the maximal fall of about 35 mm. is reached.

This is illustrated by the following two experiments:

DOSE OF CAFFEIN AT EACH INJECTION	FALL OF BLOOD PRESSURE	
	Experiment C, 111	Experiment C, 113
<i>mg.</i>	<i>mm.</i>	<i>mm.</i>
2	7	
5	12	12
10	30	18
20	30	35

The *rate of injection*, however, has even much more influence on the concentration and therefore on the acute fall:

EXPERIMENT C, 114	DOSE OF CAFFEIN	FALL OF BLOOD PRESSURE	DOSE	FALL
	<i>mg.</i>			
Very fast injection.....	3	20	20	35
Moderately fast.....	2	8	20	15
Slow injection.....	5	4	20	0

On the other hand, it matters very little into which vessel the injection is made, as shown by experiments in which the same dose of caffeine (20 mg.) was injected with nearly equal speed into various vessels (usually controlled by several injections):

EXPERIMENT NO.	FALL OF BLOOD PRESSURE WITH INJECTION INTO		
	Femoral Vein	Jugular Vein	Cephalic End of Carotid Artery
	mm.	mm.	mm.
131.....	8		8
132.....	10	10	
133.....	20	20	
134.....	35	40	
135.....	15	15	

Considering the importance of the cardiac factor, it is rather surprising that the effects are just as great on femoral and carotid injection, as when made directly into the jugular vein. Evidently, the caffein does not undergo much extra dilution on its first passage through these vascular areas.

Division of the vagi does not influence the fall.

In conclusion, then, *the second period is characterized by a rapid fall of blood pressure, in which cardiac depression predominates, probably aided by peripheral vaso-depression.*

It is worthy of incidental notice that similar falls of blood pressure occur on the intravenous injection of very many drugs, and occasionally even with saline injection.¹³ We have also found indications that the acute curare fall is predominantly cardiac. In a previous paper (Amer. Journ. of Physiology, 1910, 26:238) we concluded that the curare fall must be vascular, since it lessened the efficiency of sciatic stimulation. The force of this argument is removed by our observations on the influence of low blood pressure on vascular reactions. On the other hand, we have found that the oncometer follows the blood pressure, indicating that the curare fall is cardiac. We are following up these investigations, but take this opportunity to correct our tentative conclusion.

These falls are currently attributed to "endocardial irritation," whatever these may mean. Until the general phenomenon has been cleared up, it is not possible to say in how far an acute fall of this kind is specific for a drug.

Swirski, '04, concluded that the fall would not be cardiac, because it is not observed after chloral or after section of the medulla (but he was

¹³ Sollmann and Brown: Jour. Amer. Med. Assoc. 1905, vol. 45, p. 2'0.

Further injection of 10 mg.: { Changes in blood pressure: $140-20+40-10-10=140$
 Changes in oncometer: $175-10-12+25-4=174$
 The next injection of 15 mg. however, gave the typical result..... } Changes in blood pressure: $140-30+30-20=120$
 Changes in oncometer: $174-32+16+11=169$

The rise of blood pressure and oncometer are apt to exceed the original (the pressure in about one-sixth the oncometer, in about one-third of the experiments), especially when the total dose of caffein has been moderate (see fig. 3). In these, therefore, a

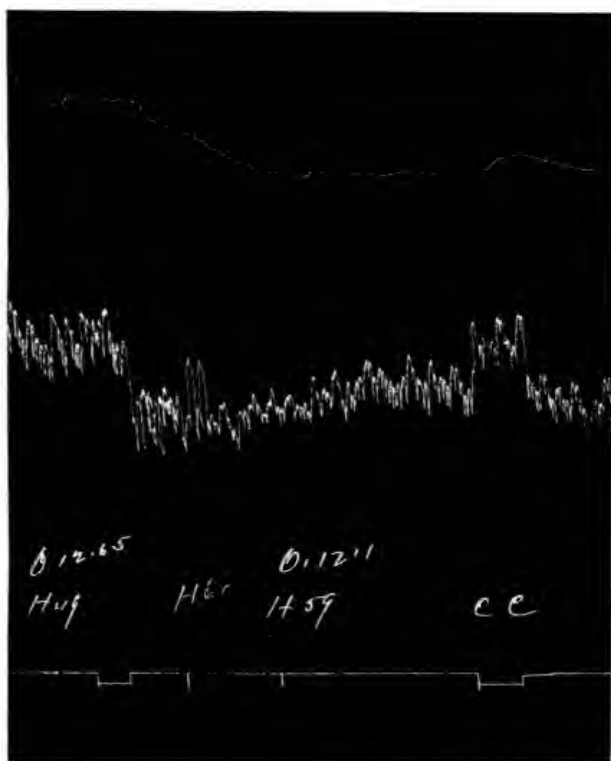


FIG. 6. INTRAVENOUS INJECTION OF CAFFEIN AFTER LARGE DOSES HAVE BEEN ADMINISTERED (EXPERIMENT C, 13-43)

Upper tracing, spleen oncometer, lower mercurial manometer. *H*, heart rate in ten seconds, *O*, oncometer reading. The animal had received 80 mg. of caffein per kg; at the first signal further 20 mg. were injected into the jugular vein, the second signal (*CC*) marks the occlusion of both carotids. The oncometer tracing is of the type "O 6."

fair degree of actual cardiac stimulation must be assumed. After large doses have been given, the blood pressure recovers but imperfectly, and the oncometer still less perfectly (fig. 6); this may be explained by cumulative depression of the heart.

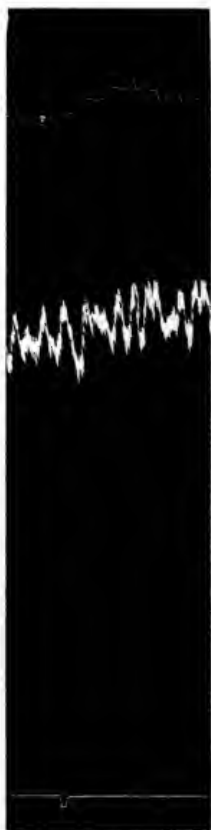


FIG. 7. CAFFEIN ON SPLEEN ONCOMETER (UPPER LINE) AND BLOOD PRESSURE

Injection of 5 mg. of caffein after previous administration of 5 mg. Oncometer is of type "Co."

THE FOURTH PERIOD

When, with the end of the third period, the abrupt rise of blood pressure is completed, the changes occur more gradually, and assume various directions. These may perhaps be best classified

according to the behavior of the oncometer: (*a*) In nearly half the cases ($\frac{23}{46}$), the oncometer rises, generally as a direct continuation of the rise of the third period. The blood pressure may also continue to rise (figs. 3 and 7), but in a somewhat greater number of observations, the pressure is falling (fig. 1), so that the vasodilator factor is predominant in this period.

This is further emphasized by a few cases in which the oncometer rise started only in this period, with falling blood pressure. The dilator action therefore tends to reach its maximum somewhat later than the cardiac strengthening, but in many cases they are fairly synchronous.

The vasomotor center usually again shows constriction in this period (as may be seen in the outflow record of fig. 12 of paper I). After the total dosage of caffein has reached 100 mg. per kilo, it is very exceptional that further injections produce an oncometer rise in this period; for then the vessels are probably dilated to their maximum, whilst the cardiac dilation which becomes permanent at this time (as will be shown in a later paper) must withdraw considerable blood from the organs.

b. Whilst the oncometer rises in nearly half the cases, as just described under (*a*), it falls in a nearly equal number. In the majority of these (one-third of the entire series), the fall starts in this period, and is then, almost without exception, accompanied by a fall in blood pressure. Generally the oncometer and blood pressure both descend below the original level, in other words, there is cardiac depression. This is the typical effect of the later injections (fig. 4).

c. About a fourth of the falls are continuations of the fall of the earlier periods, and with these, the blood pressure is usually stationary or rising, indicating a (central) vaso-constrictor element—see fig. 6.

The typical effects of the fourth period therefore consist in:

Passing of the cardiac stimulation, or increase of the cardiac depression (dilatation).

Strong peripheral vaso depression.

Central vaso stimulation, ordinarily ineffective.

THE FIFTH PERIOD

This usually presents merely a continuation of the phenomena of the preceding period toward a constant. In most instances, the blood pressure continues to fall toward normal, whilst the oncometer continues to rise. In other words, we see again that the vasodilation outlasts the cardiac stimulation (compare fig. 3). The vasomotor center, if still active, shows a gradual passing of the constriction.

Persistent changes at the end of the injection period. Comparing the conditions before each injection with those pertaining at the end of the fifth period (when the circulatory phenomena have reached a practically constant level), the following changes were found to persist: The *oncometer* remained above normal in a little over a third of the observations (28 times in 77 observations), in a slightly greater number (33 times) it had fallen below the original level. (In the remaining 16 observations, it had returned to normal.)

The final changes in the oncometer are therefore fairly evenly divided between increase and decrease.

Of the 28 observations in which *the oncometer remained increased*, the blood pressure was also raised in 10, unchanged in 12, lowered in 6. In the majority, therefore, the final changes of oncometer and blood pressure are not parallel, and even among those in which they were parallel, at least half showed a materially greater increase of the oncometer than of the blood pressure. *In the persistent oncometric rise, therefore, the cardiac factor plays a smaller rôle than the vasodilation.*

In the experiments in which the oncometer stood nearly normal at the end of the period, the blood pressure was also generally normal.

In the observations in which *the oncometer had fallen* below the original level, the blood pressure also fell, in most cases; it practically never rose. This shows that, *in the fall of the oncometer, the cardiac depression is dominant, with no evidence of vaso-constriction.*

The following shows the *influence of the total dosage of caffein* on the final oncometric response:

NUMBER OF OBSERVATIONS IN WHICH THE ONCOMETER WAS	PRECEDING DOSAGE OF CAFFEIN WAS		
	Small	Medium	Large
Higher.....	7		8
Unchanged.....	8	4	4
Lower.....	9	10	14

It will be seen that the proportion of cases showing *fall of oncometer* is distinctly greater after larger doses of caffein had been injected. This is even more marked in relation to blood pressure:

NUMBER OF OBSERVATIONS IN WHICH THE BLOOD- PRESSURE WAS	PRECEDING DOSAGE OF CAFFEIN WAS		
	Small	Medium	Large
Increased.....	8	5	3
Unchanged.....	10	7	11
Lowered.....	9	13	11

The persistent changes after caffein injection may therefore be summarized as: constant and pronounced vasodilation, with varying degrees of cardiac stimulation if the total dosage of caffein is small, or cardiac depression if the total dosage is large.

Results of previous investigators. Phillips and Bradford, '87, described the secondary expansion of the kidney volume after the injection of small doses of caffein (5 to 10 mg. per kilo). They found that this expansion often surpasses and outlasts the rise of blood pressure, enduring sometimes for 20 to 30 minutes. It was also observed after section of the splanchnics. Repeated doses gave less dilation. The spleen volume showed a lesser increase. The skull vessels also gave evidence of expansion.

The increased volume of the splanchnic organs has since been abundantly confirmed¹⁴ as also the fact that it is generally more pronounced and lasting than the rise of blood pressure.

¹⁴For instance Albanese, '91; Gottlieb and Magnus, '01; Loewi, Fletcher and Henderson, '05; Pearce, Hill and Eisenbrey, '10.

There is, consequently, a strong vasodilation, predominating over any cardiac factor. That there is a simultaneous cardiac stimulation, however, is indicated by the rise of kidney volume after section of the splanchnic or renal nerves (Phillips and Bradford, Loewi), and after chloral (Albanese), and also by the simultaneous increase of brain volume (Roy and Sherrington, '87, Phillips and Bradford). (The experiments by which Wiechowski, '02, attempted to show an active dilation of the cerebral vessels, are not conclusive. Alliprandi, '05, claimed that the cerebral vessels are constricted.)

Summary of the influence of the total dosage of caffèin on the response to acute injection. This may be studied by comparing the records of the early and late injections.

The earliest injections conform to the general type, except that the magnitudes of all the changes is less, corresponding to the smaller doses employed; perhaps the acute fall in the second period is relatively small, but of this we could not be quite certain.

The late injections depart from the type especially in the second period; as the total dosage of 80 to 100 mg. is reached, and as the blood pressure approaches the 65–80 mm. level, the acute fall of blood pressure tends to become smaller, whilst the acute fall of oncometer becomes relatively greater; in other words, the cardiac depression seems to become more important.

The oncometer also shows less tendency to recovery (see fig. 6) in the third and later periods.

In the terminal injections, i.e., in those immediately preceding death, the blood pressure and oncometer both fall gradually or suddenly, indicating cardiac failure.

The influence of total dosage on the oncometric response may also be seen from the following tabulation of the highest stand reached by the oncometer during each injection: In seventy-five observations, covering *the whole range of dosage*, the highest stand of the oncometer, in one period or another,

Surpasses the original level in	53 per cent
Merely returns to original level in	11 per cent
Remains below the original level in	36 per cent

Arranging these according to the total dosage of caffein, we find:

TOTAL DOSAGE OF CAFFEIN	THE ONCOMETER:	
	Rises	Remains below normal
mg.	per cent	per cent
20.....	73	27
30 to 90.....	54	23
100 and over.....	30	56
All doses.....	53	36

This shows plainly, that as the total dosage increases, oncometric rise becomes less frequent, and oncometric fall becomes more frequent.

Phillips and Bradford, '87, noted that the oncometric rise becomes less as the injections are repeated, i.e., as the total dose is increased. Loewi, Fletcher and Henderson '05 also remarked that each injection gives less expansion when the dosage of about 40 mg. per kilo is exceeded.

The lesser acute rise on repeated injection, which agrees with our experiments, is obviously due to the already existing partial dilation of the vessels, and to the increasing cardiac depression.

RELATIVE EFFECT ON DIFFERENT SPLANCHNIC ORGANS

Phillips and Bradford, '87, showed that the spleen volume is increased by caffein: and Loewi, Fletcher and Henderson, '05, demonstrated increase of the intestinal oncometer. It is often claimed, however, that the dilation in these organs is less extensive than that of the kidneys, and, therefore, that the renal dilation is specific. This conclusion would only be warranted if the quantitative comparisons were referred to the same cubic area of blood channel, which is obviously impossible. The evidence for a specific action on the renal vessels is therefore inconclusive.

An absolute index of the relative vasodilation effect is furnished by the proportion of injections which show a rise of the oncometer, and this, therefore, appears to us a fairer basis for comparison. We have therefore tabulated the oncometer changes in terms of percentage of the total number of observations on each organ:

The highest point reached by the oncometer, as compared with the level before each caffein injection, is:

PERCENTAGE OF EXPERIMENTS SHOWING	NUMBER OF OBSERVATIONS		
	(18)	(34)	(23)
	Kidney	Spleen	Intestine
Rise.....	39	47	74
Merely recovery to normal	5½	18	4
Recovery incomplete.....	55½	35	22

The stand of the oncometer at the end of the injection period compares as follows:

	NUMBER OF OBSERVATIONS		
	(27)	(31)	(19)
	Kidney	Spleen	Intestine
Per cent rise	30	30	58
Per cent recovery	20	30	0
Per cent incomplete recovery.....	50	40	42

Another and perhaps even more conclusive criterion of the relative vasodilator effect is furnished by the comparison of the duration of the rise in oncometer and blood pressure, for if the oncometer remains high after the blood pressure falls, this means that the local dilator factor is more powerful than the general cardiac factor.

Our records show the following results:

PERCENTAGE IN WHICH THE ONCOMETRIC RISE WAS	NUMBER OF OBSERVATIONS		
	(19)	(15)	(3)
	Kidney	Spleen	Intestine
More persistent than the blood pressure rise.....	22	33	66
Approximately the same as the blood pressure rise.....	63	66	33
Shorter than the blood pressure rise.....	15	7	0

These figures certainly give no warrant to the belief that the dilator action of caffein is especially strong on the kidney; in fact, the kidney and spleen behave essentially alike, whilst the intestine appears to show the strongest dilator action. We would hesitate, however, to attach much importance to the last conclusion until it has been investigated how the intestinal musculature reacts to caffein.

The reputed vaso-constrictor action of caffein. The older writers believed that caffein produces a powerful vasoconstriction throughout stimulations of the vasomotor center. This belief still prevails widely, although it was originally based upon very slender and indirect evidence. It was only with the introduction of the oncometer that positive data became available, and these show conclusively that the moderate rise of pressure which is observed in anesthetized animals is in most cases exclusively cardiac. By our perfusion method, with nerve connections intact, we have brought direct evidence that the vasoconstrictor center is indeed stimulated, but this stimulation is rarely effective against the peripheral vaso-depressor action of the caffein. The dominant action on the vessels is therefore in the direction of dilation, instead of constriction. In exceptional conditions however, the central constriction may overcome this peripheral depression; as when the vasomotor center has been depressed by partial asphyxia, or when the animal awakens from the narcosis and struggles, etc., but these cases are rare exceptions.

The vasoconstriction-theory was challenged by Albanese, '91, on the basis of the oncometer results. The weakness of the vasoconstrictor evidence is thoroughly discussed by Loewi, '05. The theory seems to have originated in the observation of Wagner, '85, that caffein did not raise the blood pressure after large doses of chloral. He assumed that this had paralyzed the vasomotor center, but his own experiments, according to Loewi, bear evidence that the vasomotor center was not paralyzed, since it still responded to asphyxia. Wagner's evidence has now lost all force, since it has been shown conclusively that caffein may raise the blood pressure after sections of the cord or of the splanchnics, and that the lessened response under these conditions may be attributed simply to the lowered blood pressure.

Von Schroeder, '86, assumed vasoconstriction as an easy explanation of his observation that caffeine is not usually diuretic in morphinized rabbits, but that it is always effectively diuretic in chloralized rabbits. In his next publication ('87), however, he describes that paraldehyde favors the diuresis, just as does chloral, although the blood pressure was not lowered, and therefore, the vasomotor center was certainly not paralyzed. Evidently then, if the morphinized rabbits really showed vasoconstriction, this must have been due to the morphine, and not to the caffeine.

Phillips and Bradford, '87, obtained results with the oncometer which, in fact, disproved the vasoconstrictor theory, but they failed to grasp their significance.

Reichert, '90, and Swirski, '04 accepted the vasoconstrictor theory without any further evidence.

PRINCIPAL TYPES OF BLOOD-PRESSURE AND ONCOMETER CHANGES

To comprehend the relations and the relative frequency and importance of the changes which have been described, it seems advantageous to present them diagrammatically. In this way, it was found that the majority of the otherwise complex and confusing tracings could be reduced to a few principal types, as shown in fig. 8. The records of 57 injections were used in this analysis.

Oncometer Type A (see figs. 1 and 3). In this type, the oncometer rises continuously through the third, fourth and fifth periods, reaching a level generally considerably above the original. This occurs in 40 per cent of the injections, and is the most frequent type when the total dosage is below 100 mg., and may be considered the characteristic type for the earlier injections.

The blood pressure curves correspond mainly to types 1 and 2, some to type 3. That is, the blood pressure tends to rise (cardiac stimulation) but to a considerably less degree than the oncometer (vasodilation).

Oncometer Type C (see fig. 7). After rising in the third period, the oncometer starts to descend in the fourth period, but usually, does not fall to its original level. This occurred in 14 per cent of the injections. In most of these, the blood pressure follows

the oncometer fairly accurately (type 2-cardiac stimulation) but in a few, the pressure fell more rapidly than the oncometer (vasodilation).

Oncometer Type K (see fig. 4). In this, the oncometer starts to recover from the primary fall, but remains distinctly below the

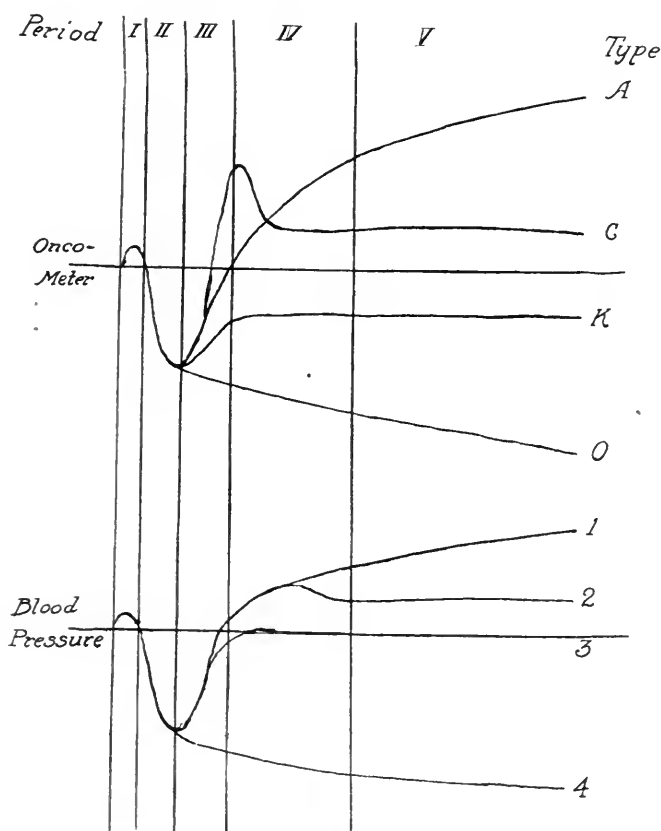


FIG. 8. DIAGRAM OF THE ACUTE ONCOMETER AND BLOOD PRESSURE PHENOMENA, WITH CAFFEIN INJECTION

original level. With its minor variations, this type comprises 37 per cent of the injections. It is especially common when the total dose of caffein has been fairly large, and it may be considered the characteristic type for the later injections.

The blood pressure usually approaches the type 3; i.e., it recovers rather better than the oncometer. This shows that with this late type, there is little further dilation.

Oncometer Type O (see fig. 6). The oncometer does not recover, but continues to fall, as also the blood pressure (type 4). This type (which was not counted in the above 57 records) occurs usually at the immediate approach of death.

SUMMARY

The intravenous injection of caffeine produces the following acute effects:

1. A slow and slight rise of blood pressure and oncometer, referable to the acute influx of fluid. This is followed by

2. A sharp fall of blood pressure and oncometer, indicating acute cardiac depression. The oncometer and respiratory centers are somewhat stimulated by anemia. The heart rate is quickened. There is also some indirect evidence of peripheral vaso depression.

The further course depends upon the total dosage of caffeine:

- 3a. With the earlier injections, corresponding to *small and moderate dosage*, the blood pressure rises somewhat above the original level. The oncometer shows a greater and more persistent rise. There is consequently cardiac stimulation and simultaneous vasodilation—the latter being the more persistent. Very exceptionally there is a central vaso-constriction.

- 3b. With the later injections, corresponding to *larger dosage*, both the blood pressure and especially the oncometer recover only imperfectly. The cardiac depression therefore tends to become persistent, and the additional vasodilation becomes less (the vessels being already permanently dilated).

- 3c. At the *immediate approach of death*, the blood pressure and oncometer continue to fall, indicating terminal cardiac paralysis.

PRACTICAL APPLICATIONS OF THE CIRCULATORY ACTIONS OF
CAFFEIN

The circulatory effects of moderate doses of caffein consist in vasodilation combined with sufficient cardiac stimulation to maintain or even somewhat to increase the blood pressure. Both of these actions favor blood flow, a result which must be highly desirable in many *circulatory diseases*. The actions of caffein are, however, quite different from those of digitalis, in that its cardiac actions are "stimulant" rather than tonic, brief and less powerful; the action on the vessels is diametrically the opposite, caffein acting as a dilator, while digitalis tends to constrict.

The main therapeutic use of caffein, namely as a diuretic, is also explainable on the basis of its circulatory actions, i.e., by the increased blood flow to the kidneys, which furnishes the best possible condition for the formation of urine. The experiments of Phillips and Bradford, '87; Gottlieb and Magnus, '01; Loewi, Fletcher and Henderson, '05; Pearce, Hill and Eisenbrey, '10, and others, all show a very satisfactory correspondence of the urine flow with the changes of the renal circulation, as recorded by the oncometer. The correspondence may not be absolute in individual experiments, but such exceptional divergences are to be expected, and may be attributed to accidents, to technical imperfection, and to the occasional predominance of the central vasoconstrictor factor. The common view that caffein acts directly on the epithelium has no better foundation than the fancied need to explain these exceptional variations. There has never been any good positive evidence for the assumed direct stimulation of the renal epithelium. This theory was originally advanced by von Schroeder because the blood pressure changes fail to account for the diuresis. This is now explained by the double action of caffein on the heart and vessels, and v. Schroeder's arguments are thus deprived of their logical basis.

This does not exclude the possibility of a secretory stimulation as an additional factor; but it is not justifiable to assume this without some evidence—and such evidence is still practically lacking.

The circulatory explanation of the diuresis does not mean that the kidney play a wholly positive part. Caffein will not increase the urine flow if the kidney structures are severely injured, particularly the glomeruli; or if there is not sufficient liquid at the disposal of the body, or if the diuresis is already very free. This is merely equivalent to saying that the caffein does not cause urine secretion, but that its actions on the renal vessels and on the heart result in conditions of the renal circulation favorable to urine formation.

The circulatory actions of caffein may also become detrimental. Our experiments furnish definite evidence of cardiac injury when the dosage exceeds 20 to 40 mg. per kilo. These doses are larger than those ordinarily taken by man, but the differences are not so very great, and the cardiac irregularities which are so often seen in man are evidence that smaller doses may be injurious in susceptible individuals.

The gastro-intestinal disturbances which are an important feature of chronic caffein poisoning may possibly be attributed to its vasodilator action. With the beverages, the tannin of tea or the empyreumatic oil of coffee are doubtless factors—and perhaps the more important factors—in these local lesions, but Salant and Rieger, '10, found that the continuous administration of pure caffein to animals resulted in intestinal and especially gastric inflammation.

Translation of dosage. To obtain some idea of the significance of these facts, it may be remarked that, judging from the fatal dose, different animals behave quantitatively nearly alike. It is therefore probable that the dosage can be transferred to man:

1 mg. per kg. would correspond to 1 grain for a 145 lb. (65 kg.) man
1½ grain would correspond to 5 mg. per kg. for a 44 lb. (20 kg.) child
20 mg. per kg. would be 20 grains for 145 lb. man (=abt. 1.5 gms.)
6 grains for 45 lb. child (=abt 0.4 gm.)
140 mg. per kg. would be 140 grains for man = abt. 10 gms.
43 grains for child = abt. 3 gms.

CONCLUSIONS

1. The effect of caffein on the circulation are shown to involve the following *factors*:

Cardiac stimulation or depression, according to the dose and rapidity of injection.

Increased heart rate, not due to vagus depression (if the vagus is intact, there may be slowing through central stimulation, but with large doses, the heart escapes from the vagus tone).

Vasodilation, through peripheral depression of the vasoconstrictor mechanism.

Central vasoconstrictor stimulation generally ineffectual, also convulsive stimulation.

Cardiac irregularities, with large doses.

2. *The early intravenous injections* show the following phenomena:

Momentary myocardial depression, succeeded by myocardial stimulation.

Peripheral vasodilation.

Central vasomotor stimulation, usually ineffective.

Increased heart rate.

Blood pressure: primary fall, followed by recovery and often a small rise.

Oncometer: primary fall, followed by larger rise, usually outlasting that of the blood pressure.

3. *Summation of effects with cumulative doses*:

a. Total dosage of 20 to 150 mg. per kilo shows:

Cardiac depression (dilatation).

Peripheral vasomotor paralysis.

Moderate and ineffective central vasomotor stimulation.

Heart rate reaches maximum and becomes irregular.

Blood pressure falls to constant level of 50 to 70 mm.

Fall of oncometer.

b. The blood pressure reaches a constant level when the dosage reaches about 150 mg. per kilo, and further doses of caffein may than be injected with little effect, but sudden cardiac failure may supervene at any time.

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A CONSIDERATION OF SOME BIOLOGIC TESTS FOR EPINEPHRIN

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In connection with other researches during the past year a series of investigations has been made of the comparative utility of certain biologic tests for epinephrin. Of those hitherto described the frog's eye, uterus, and intestinal strip methods seemed most promising and were selected for further investigation.

I. THE FROG'S EYE TEST

The Meltzer-Ehrmann reaction has the disadvantages of lack of sharpness, and insufficient delicacy for many researches. By applying the fluid to be tested directly to the iris, however, a sharpness of reaction was obtained considerably greater than that following the application of the fluid to the corneal surface. This procedure involves considerable manipulative difficulty but by the following technique successful results were secured without undue consumption of time: the frog is decapitated by a quick cut with strong scissors passing through the cranium near the angle of the jaw. The eyes are then removed and freed from adherent tags of tissue. During these manipulations undue pressure upon the eyeball must be avoided. Through the stump of the optic nerve a fine scissors point is inserted and a small incision made so that the sclera may be grasped by a pair of forceps; this grasp is retained while the whole eye is circumsected about a millimeter posterior to the margin of the iris. The lens now lies in a cup formed by the anterior half of the eye from which it must be removed without injury to the pupil. This can be done

by holding down the eye with a pair of forceps between the tips of which the lens is grasped by a second pair and gently removed. The pupils of pairs of eyes so prepared are usually approximately equal in size, and one can be used as a control for the other. The eyes should, of course, be placed in Ringer's or isotonic saline solution until ready for use but they should be not kept longer than fifteen minutes before being used. Receptacles made by cementing segments of glass tube 1 cm. long and 1 cm. in diameter to ordinary wide microscopic slides are convenient, particularly if the pupils are to be observed under the microscope. The delicacy of the test is enhanced by following Schultz¹ suggestion to keep the eyes in the dark except during observation.

By this method a mydriasis is secured unmistakably evident by inspection within five minutes in a dilution of adrenalin, 1:5,000,000. By means of camera lucida tracings at a magnification whereby slight differences in the size of the pupils can be distinguished, it is possible to determine adrenalin in considerably higher dilution; the upper limit is about one part in a hundred millions. This dilution requires from ten to twenty minutes' application. In a trial series of eight determinations of adrenalin, 1:100,000,000, at a magnification of twenty-five diameters at the end of fifteen minutes six were positive. The average increase in diameters was: longitudinal, 11 per cent; transverse, 25 per cent, greater in cases of the eyes in adrenalin than of control eyes in isotonic saline solution.

For use in studies of epinephrinemia the pupil reaction is not sufficiently specific. The mydriatic effect of epinephrin is shared by pituitary extract,² parathyroid neucleo-protein and iodothyron.³ Extract of desiccated thyroid gland also was observed to cause dilatation.

The chief advantage of the method is that it can be carried out with a small quantity of fluid.

¹ Schultz: Bulletin No. 61, Hygienic Laboratory, United States Public Health and Marine Hospital Service, Washington, 1910.

² Cramer: Quarterly Journal of Experimental Physiology, 1908, I: 189; Ott and Scott: New York Medical Journal, 1908, lxxxviii: 1180.

³ Ott and Scott: Monthly Cyclopedia and Medical Bulletin, 1909: ii: 493.

II. THE UTERUS TEST

Fränkel's statements as to the advantages of his uterus method⁴ were readily confirmed. By his technique reactions to adrenalin were obtained in dilutions of one to twenty or thirty millions. There are however, certain material objections to the method. The uterus is, as Fränkel himself has noted, particularly sensitive to mechanical stimulation, and even with careful manipulations is somewhat erratic in its reactions. Its tendency to undergo spontaneous rhythmic contractions in Ringer's solution precludes its use for determination of minimal quantities of epinephrin; with larger quantities, however, spontaneous and induced contractions are easily differentiated. So far as its use in studies of epinephrinemia is concerned, a more serious disadvantage is, as in the case of the frog's eye, a lack of specificity. Ott⁵ has observed that the tendency of adrenal extract to cause contractions of uterine segments is shared by extracts of mammary, prostate, parotid, pancreatic and pituitary glands, spleen, thymus, testicle and ovary, and by spermine and iodothyryn. Moreover, by direct experiment it was found that rabbit's blood taken an hour after epinephrectomy causes a marked contraction. Fränkel's conclusion therefore, that any tendency of an experimental blood to cause contractions in the uterus is due to the presence of epinephrin is quite unjustified.

III. THE INTESTINE TEST

An attempt to adapt the intestinal strip method⁶ to the rabbit led to the discovery that segments of small intestine serve admirably as biologic tests for epinephrin. The technique employed was similar to that of other investigators in this field. Pieces 3 or 4 cm. long are removed from the animal to warm oxygenated Ringer's solution in which almost immediately rhythmic contractions begin and continue for hours. To permit free access of oxygen the

⁴ Fränkel: *Archiv für experimentelle Pathologie und Pharmakologie*, 1909, lx: 395.

⁵ Ott: *Journal of Experimental Medicine*, 1909, xi: 326.

⁶ Cannon and de la Paz: *American Journal of Physiology*, 1911, xxviii: 64.

pieces are suspended by glass hooks through the mesentery. From time to time as required, segments can be used to obtain graphic records. The piece is attached to a writing lever and suspended in Ringer's solution in a cylindrical container surrounded by water at 37° C. Oxygen is constantly bubbled into the cylinder from a supply tube connecting with the base. The lower end of the tissue is held by a *serre-fine* attached to a thread leading out through the supply tube. A second small tube connecting with the base of the cylinder and with a piece of rubber tube at its free end facilitates the changing of fluids in the container with a minimal disturbance of the segments. The animal from which the intestine is taken should not be etherised since the activity of the segments is thereby much diminished. The animals can be killed by a blow on the neck or anaesthetized with urethane *per os*, 2 grams per kilo. This substance does not share the depressing effect of ether upon the activity of the intestine. The intestine with its normal blood supply maintains its vitality better than if kept in Ringer's solution and during the long anaesthesia following administration of urethane, segments can be removed as required.

The observations of Ott,⁷ Magnus,⁸ Boruttau,⁹ Langley,¹⁰ Pal,¹¹ Elliott,¹² and Cannon and de la Paz⁶ that epinephrin inhibits the activities of the intestine was readily confirmed. It was found indeed that the threshold of this effect is surprisingly low. In the most favorable instances immediately after removal of the tissue from the animal, a brief but clear cut diminution of activity was secure in dilutions of adrenalin¹³ of from four to five

⁷ Ott: Medical Bulletin, 1897, xix: 376.

⁸ Magnus: Archiv für die gesamte Physiologie, 1905, cviii: 50.

⁹ Boruttau: Ibid., 1899, lxxviii: 97.

¹⁰ Langley: Journal of Physiology, 1901, xxvii: 249.

¹¹ Pal: Wiener medizinische Presse, 1901, xlii: 2023.

¹² Elliott: Journal of Physiology, 1905, xxxii: 401.

¹³ For these experiments a fresh bottle of adrenalin was secured directly from the Boston agency of Parke, Davis and Company. This brand of epinephrin has been shown by Schultz¹ to test quantitatively true to label. The fact that it caused reactions in rabbit's uterus at a dilution of one to twenty or thirty millions as a minimum indicates that the sample used did not surpass its supposed strength.

hundred millions (Cf. Fig. 1). Fig. 2 shows a characteristic record of the effect of adrenalin in dilution of 1:100,000,000. This diminution in amplitude of oscillation is usually accompanied by a decreased tonus. The threshold of this effect varies *pari passu* with the vitality of the tissue. In some instances after keeping the segments three or four hours in Ringer's solution reactions were not secured in higher dilutions than one in five millions. Either Ringer's solution or defibrinated blood may be used as a diluent, but in the former case, similar blood must of course be used as a standard. The degree of depression of the tissue depends directly upon the strength of solution employed. A sensitive tissue can be brought to a complete stand-still by a dilution of one to twenty or thirty millions. The sensitiveness of the tissue to epinephrin diminishes to some extent after each application but a number of successive records can be secured with one segment. This fact permits comparing an unknown solution and a known standard, with some degree of ease. If the order of application of standard and "unknown" be varied the method is capable of giving valuable quantitative results. Segments of cat's intestine are about as sensitive as rabbit's but the amplitude of oscillation being smaller the results are less striking. In cat's blood, rabbit's intestine beats as freely as in that of the rabbit itself. Other bloods were not tested.

The use of intestinal segments has several distinct advantages. They are always available and with the possible exception of artery strips¹⁴ are the most sensitive test objects yet described. The intestine has the advantage over both the uterus and the artery that it will respond to a number of applications of epinephrin, and hence can be used with greater certainty in quantitative work. It has the obvious advantage over the intestinal strip method that the tissue is subjected to much less manipulative violence and is consequently in much more sensitive condition. Both the intestinal methods have the advantage of greater specificity than either the frog's eye or the uterus methods; there are few known substances occurring in blood that cause dilatations

¹⁴ Cf. Meyer: *Zeitschrift für Biologie*, 1907, 1: 93.

of smooth muscle.¹⁵ It is to be noted, however, that the blood of an animal dying in asphyxia gives the depression reaction with intestinal segments even if the adrenals have been previously extirpated. So far as the writer is aware, no other gland shares with the adrenals their depressing effect upon the gut. Ott and Scott¹⁶ found that intestinal peristalsis is even increased by iodothylin, ovary, infundibulin, mammary gland, prostate, thymus, spleen, parathyroid, pancreas, dried brain and parotid gland. The material in each case was extracted and injected in aqueous solution by jugular. Peristalsis was observed by connecting a balloon inserted into the intestine with a piston recorder. By the intestinal segment method these investigators found that pancreatic extract sometimes showed a tendency to relax the gut and slow rhythmic contractions, but a repetition of their work has shown that the relaxing effect is obtained only if the pancreas has been kept for a time; if the extract is made immediately after the death of the animal, it increases the contractions. The depression observed was probably due, therefore, to digestive products. Blood in itself, as Cannon and de la Paz,⁶ have noted, has a marked tendency to increase tonus in the gut, and the depression reaction, when secured in a blood of unknown composition, has accordingly the more significance as indicating the presence of epinephrin. The fact that the segments usually begin activity immediately after attachment to the writing lever adds not a little to the satisfaction of the method for routine work. It seems, therefore, that in point of specificity, promptness and delicacy, the intestinal segments are the most satisfactory test objects for epinephrin yet described.

I wish to express my appreciation of the permission to make use of the facilities of the Laboratory of Physiology in the Harvard Medical School in this research.

¹⁵ Grützner: *Ergebnisse der Physiologie*: 1904, iii (2): p. 66.

¹⁶ Ott and Scott: *American Medicine*, 1911, xvii: 154.

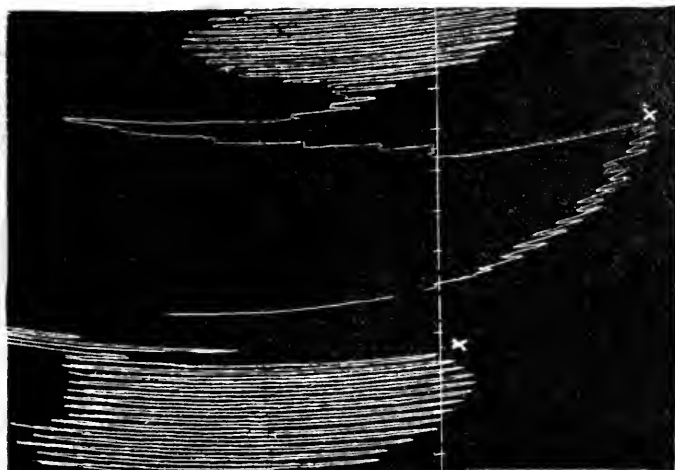


Fig. 2. Rabbit's intestine in Ringer's solution.
At X Ringer's solution + Adrenalin 1:100,000,000;
At X' Ringer's solution substituted. Time, 30
sec.

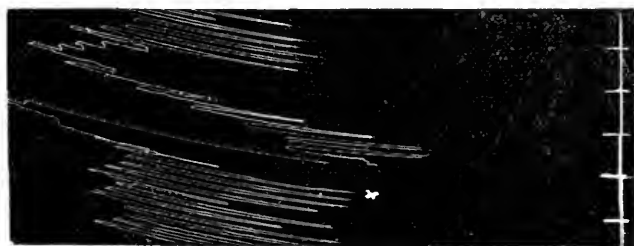


Fig. 1. Segment of rabbit's intestine beating
in defibrinated blood of epinephrectomised rabbit.
At X blood substituted by similar blood contain-
ing adrenalin 1:400,000,000. Time, 30 sec.

STUDIES ON THE PHARMACOLOGICAL ACTION OF OXIDIZING SUBSTANCES¹

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The only two fundamental processes occurring in living things of which any considerable amount of knowledge has been accumulated are processes of oxidation and hydrolysis. Under the term oxidation we include reduction because every oxidation implies a reduction and under the term hydrolysis is included the reverse process, namely, hydrosynthesis.

In spite of the fact that processes of oxidation play such an important rôle in the life of every living cell yet we have, at present, little knowledge of the effect of increased physiological oxidation on the functions of cells or tissues. The ability of oxygen to increase oxidation in higher animals is limited by the oxygen carrying power of the blood.

In arterial blood the haemoglobin is almost saturated with oxygen and the increase in the oxygen in the blood when the pure gas is breathed is very slight. The work with ozone and the alkalis has likewise not thrown very much light on the reaction of cells to increased oxidation.

Pharmacological studies of the action of many of the more violent and destructive oxidizing agents have been made. Most of these cannot be used intravenously. Many cause methaemoglobin formation and hydrogen peroxide when injected intravenously causes death by gas embolism. Many of these substances, such as the chlorates, are not reduced in their passage through the body, from which we may conclude that their oxygen is not

¹ The expense of this investigation has been partially defrayed by a grant from the Rockefeller Institute for Medical Research.

available for physiological oxidation. Physiological oxidations are brought about through the agency of the oxidases. Kastle and Loevenhart² showed that the organic peroxides in general have many of the characteristics of certain of the oxidizing ferments and they were led to agree with Bach³ that the production of organic peroxides in the cell would readily account for much that is known regarding vital oxidation. In 1905 Loevenhart⁴ published a short paper on the therapeutic uses of benzoyl peroxide wherein it was shown that this substance is an ideal local antiseptic.

We were led to believe in the course of this work that were it possible to obtain a soluble preparation of the type of benzoyl peroxide it would prove of great value as an internal antiseptic which could be administered intravenously. In the second place, we were anxious to investigate the pharmacological action of some substance which could be injected intravenously and which, from its chemical nature, might be supposed to increase the normal physiological oxidation of cells.

Several substances are known which interfere with vital oxidation without apparently having any other effect, and cells may readily be subjected to conditions in which their processes of oxidation are inhibited. For these reasons we are well acquainted with the phenomena accompanying a decrease in the vital oxidation. On the other hand, it has thus far been impossible to study the effect of increased oxidation in living tissues and accordingly the investigation of the physiological response to increased oxidation is of the most vital importance to physiology and pharmacology.

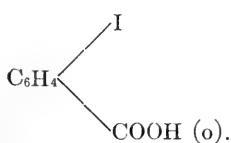
It was in the hope of throwing light on these two propositions that we undertook the study of compounds containing oxygen in a form which might be available physiologically. Up to the present time we have investigated the action of only three such

² Amer. Chem. Jour., 26, 539 (1901).

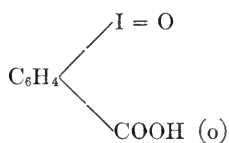
³ Compt. rend., 124, 951 (1897).

⁴ Therapeutische Monatshefte., 19, 426, (1905). The views here expressed regarding the therapeutic value of this drug in the conditions mentioned have since been confirmed by a great many clinical observations in the hands of a number of men.

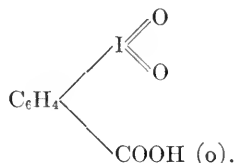
substances, namely, sodium iodosobenzoate, sodium iodoxybenzoate, and the sodium salt of phthalic peroxide. In the present communication we shall merely present the studies of the first two substances. Throughout the work we have studied the effect of sodium iodbenzoate, the mother substance as a control. The formulas of the three acids are as follows:⁵



Iodbenzoic acid



Iodosobenzoic acid



Iodoxybenzoic acid

All of these acids when perfectly pure are white or practically so. When nearly pure they have a faint cream color. Ortho-iodobenzoic acid has been long known. Iodoso- and iodoxybenzoic acids were first prepared by Victor Meyer and his coworkers.⁶ The free acids are insoluble in water but the sodium salts of all are very soluble. The principal interest connected with the compounds centers around the oxygen combined with the iodine. This oxygen is readily liberated in consequence of which iodoso- and iodoxybenzoic acids are capable of oxidizing many substances. Iodbenzoic acid of course has no oxidizing action. Iodoso- and iodoxybenzoic acids when reduced yield iodbenzoic acid. These two acids oxidize hydriodic acid quantitatively and the liberated iodine can be estimated readily by titration with $\frac{N}{10}$ sodium thiosulphate using starch paste as the indicator. This, together with the melting points, affords a ready method of determining the purity of these substances and all material used in our work was analysed in this way. The calculated amount of active oxygen contained in these substances is as follows:

	<i>Per cent</i>
Iodbenzoic acid.....	0.00
Iodosobenzoic acid.....	6.06
Iodoxybenzoic acid.....	11.43

⁵ There are other views regarding the structure of iodosobenzoic acid but it is unnecessary to consider them in connection with our work.

⁶ Meyer and Wachter: *Berichte d. d. chem. Ges.*, 25, 2632 (1892). Askenasy and Meyer: *Berichte d. d. chem. Ges.*, 26, 1354 (1893). Hartmann and Meyer: *Berichte d. d. chem. Ges.*, 26, 1727 (1893); 27, 1600 (1894).

It is interesting and important in the light of the pharmacological action of these substances to compare their strength as acids. Ortho-iodbenzoic acid is the strongest of the three iodbenzoic acids. Iodosobenzoic acid is extremely weak. Its salts are decomposed by carbon dioxide and the acid precipitated. Iodoxybenzoic acid has marked acid properties. Askenasy and Meyer⁷ determined the dissociation constants of iod- and iodosobenzoic acids, which were found to be as follows:

Iodbenzoic acid.....	k. = 0.132
Iodosobenzoic acid.....	k. = 0.00006
Carbonic acid.....	k. = 0.0013

The dissociation of iodoxybenzoic acid has apparently not been determined. In aqueous solution it has a very sour taste. It decomposes carbonates with liberation of carbon dioxide and on passing a current of carbon dioxide through a solution of barium iodoxybenzoate there is no precipitation of barium carbonate.

A word as to the methods which we have used in preparing these substances may not be amiss, although we have not departed very widely from the methods given by Meyer and his coworkers. We have compared the various methods which they used both as to the purity of the products and the yield.

Preparation of iodbenzoic acid: Suspend 20 grams anthranilic acid in water and add 50 cc. concentrated hydrochloric acid, warm to bring into solution, then cool to below 10°. Add 10 grams sodium nitrate in solution and after about five minutes add 30 grams of potassium iodide in solution. When the reaction is nearly over the contents of the flask are heated on the water bath. The buff colored precipitate of iodbenzoic acid is filtered off on a perforated plate with suction and dried. The yield is from 1¼ to 1½ times the weight of the anthranilic acid employed. This crude product is sufficiently pure to prepare the crude iodosobenzoic acid. For pharmacological work it can be purified in various ways; by dissolving in alkalis and precipitating with acid or by recrystallization or sublimation. In our hands the simplest way of procuring a perfectly pure white product in sufficient

⁷ Berichte d. d. chem. Ges., 26, 1354 (1893).

quantities is to transform it into pure iodosobenzoic acid and then reduce this to iodbenzoic acid. This yields a beautiful product.

Preparation of iodosobenzoic acid: Thirty grams of crude iodbenzoic acid, 700 cc. of 2.3 per cent potassium permanganate, 270 cc. 20 per cent sulphuric acid and 180 cc. of water were boiled together for fifteen minutes. Then boiling water was added until the total volume was 2 liters. The boiling was then continued for fifteen minutes, filtered through a hot water funnel and the iodosobenzoic acid which crystallized out on cooling was filtered off, dried, weighed and analysed. The yield is about gram for gram of the iodbenzoic acid used. The material contains from 5.88 per cent to 5.90 per cent of active oxygen. This crude product is sufficiently pure for the preparation of iodoxybenzoic acid. To obtain a pure product, the crude acid is dissolved in sodium hydroxide, a great excess being avoided, filtered and precipitated by passing a current of carbon dioxide into the solution. A second, and frequently a third precipitation of iodosobenzoic acid may be obtained by passing more CO_2 through the filtrates. A beautiful product is invariably obtained by this method. It is almost perfectly white. The crystals are small, due to the agitation of the solution. The yield is good, the loss being less than 10 per cent. This product invariably has a content of 6.03 to 6.06 per cent of active oxygen. This method of preparation is far superior to the first method proposed by Meyer and Wachter in which the iodbenzoic acid was oxidized by means of fuming nitric acid. This method of purification is the best we have been able to devise and leaves little to be desired.

Preparation of iodoxybenzoic acid: We have employed the chlorine method of Hartmann and Meyer. This consists in passing a current of chlorine through an alkaline solution of sodium iodosobenzoate, keeping the temperature down by immersing the bottle in cold water. Meyer and Hartmann give directions for separating the iod-, iodoso- and iodoxybenzoic acids which are the products of the reaction. We have found that crude iodosobenzoic acid can be employed and that by carefully controlling the temperature at which the chlorine acts, it is possible to obtain at once almost pure iodoxybenzoic acid. It may

be rendered perfectly pure by a single recrystallization. The temperature at which the chlorine acts must be neither too high nor too low. Considerable heat is generated in the process and if the reacting mixture becomes too warm the product will be worthless. If the mixture is kept too cold considerable quantities of iod- and iodosobenzoic acids will be found mixed with the iodoxybenzoic acid. We have obtained the best results by filtering the solution before passing the current of chlorine through it and by immersing the bottle in which the oxidation occurs in tap water at from 15° to 20° and towards the end of the process adding warm water to the bath to bring its temperature up to 30° or 35°. The yield of iodoxybenzoic acid is about 75 per cent of the crude iodosobenzoic used.

THE PHARMACOLOGICAL ACTION OF THE THREE ACIDS AND OF THEIR SODIUM SALTS

As far as we have been able to find the action of iodoxybenzoic acid on living organisms has not been studied. No particular attention has ever been paid to the action of iodbenzoic acid.

The only study of the action of iodosobenzoic acid which we have been able to find is that of R. Heinz.⁸ He studied iodosobenzoic acid with the view of investigating the action of nascent iodine in the body, thinking that by giving potassium iodide by mouth and sodium iodosobenzoate locally, iodine would be liberated in the body in the nascent state. In speaking of sodium iodosobenzoate he says: "Aus diesem Grunde ist es für Thierversuche nicht verwendbar, indem es äusserst heftig reizt, das Blut rasch auflöst und bräunt, die Gewebe aufquellen und, soweit sie bluthaltig sind, missfarben macht." Heinz described his preparation of iodosobenzoic acid as consisting of yellowish leaflets. This would indicate that he used the fuming nitric acid method described by Meyer and Wachter, in preparing it.

He states that the sodium salt is strongly alkaline and for this reason is not available for animal experiments. In his experiments he therefore employed only the slightly soluble free iodo-

⁸ Virchow's Archiv., 155, 44 (1899).

sobenzoic acid in the form of a powder or emulsified with gum arabic or starch. Heinz found that iodosobenzoic acid when placed in the stomach of a rabbit causes hyperaemia and small haemorrhages of the gastric mucosa. This he attributes to its local irritating action, and states that the effect can also be shown by placing some of the powder in the eye (without simultaneously injecting sodium chloride). This irritating action he states is due in part to the acid nature of the substance and in part is characteristic of these compounds, all iodoso compounds even in neutral solution being more or less irritating.

Heinz also studied to some extent the fate of iodosobenzoic acid in the body. He showed that none escapes in the urine as iodosobenzoic acid nor as iodosohippuric acid, because the urine does not liberate iodine from hydriodic acid. Within the first hour, Heinz found that the urine gives a distinct test for iodine on ashing while the test for sodium iodide is negative. Later he found inorganic iodides present in the urine. He states, therefore, that in the body a gradual change in the iodosobenzoic acid occurs whereby iodine is liberated and combines with the alkali in the tissue juices to form iodides. This only occurs very slowly and produces no recognizable physiological effect.

The local action of the substances: Iodobenzoic acid dusted into the eye of a rabbit causes a slight degree of congestion after twenty-four hours, but within forty-eight hours the eye is perfectly normal. Sodium iodbenzoate when instilled into the conjunctival sac or injected subcutaneously in $\frac{N}{30}$ solution is without irritating action.

Iodosobenzoic acid: Heinz found the free acid as well as the sodium salt extremely irritating. He describes the material used as having a light yellow color, whereas the pure substance is almost white. Hence we determined to study the local action of the pure substance. When the dry white powder is dusted into one eye of the rabbit (the other eye being kept as a control), the reaction is practically the same as that resulting from any bland insoluble powder. There is no redness nor injection of the vessels. The eye was not kept closed, there was no increase in the secretion, no extension of the nictitating

membrane, no change in the size of the pupil. After instilling two drops of a $\frac{N}{25}$ solution of sodium iodosobenzoate into the conjunctival sac of a young dog four times within a period of eight minutes, the eye did not show the slightest reaction and the animal apparently suffered no discomfort in any way. One of us (Loevenhart) took by mouth 0.3 gram of free iodosobenzoic acid suspended in water and three hours later 0.5 gram and $2\frac{1}{2}$ hours later 0.5 gram, a total of 1.3 grams within $5\frac{1}{2}$ hours without the slightest symptom being noted. A number of observations were made on the effect of sodium iodosobenzoate when injected subcutaneously and intraperitoneally.

A description of one experiment with the findings will suffice to illustrate its action on subcutaneous tissue.

Two cubic centimeters of a sterile $\frac{N}{30}$ (0.953 per cent) solution of sodium iodosobenzoate were injected subcutaneously on the back of a rabbit near the middle line about 6 cm. above tail. After forty-eight hours the animal was killed by a blow on the neck and examined.

Autopsy notes: There is a thickening of the subcutaneous tissues at the site of the injection which has a semifluid feel. On incision there is found a wet gelatinous infiltration of the subcutaneous tissues, extending from 2 to 3 cm. above the puncture wound around the animal nearly to the midline on the abdomen, down both thighs a considerable distance and posteriorly to a point 1 to 2 cm. below the tail. There is marked injection of the smaller vessels of the fascia and muscles, minute haemorrhages in the fascia and one quite large haemorrhage in the muscle. There is one opaque, apparently necrotic area, in the lumbar muscles below the area of the haemorrhage.

Local action in the peritoneal cavity: Fifteen cubic centimeters of various strengths of sodium iodosobenzoate from $\frac{N}{30}$ to $\frac{N}{100}$ were placed in the peritoneal cavity of rabbits aseptically. Under anaesthesia a very short incision was made in the abdominal wall down to the peritoneum. The rounded end of a fine pipette was thrust through the peritoneum and the solution allowed to flow in. No stitch was taken in the peritoneum. The abdominal muscle and skin were sewed up separately. Three days later the animals

were killed by a blow. When 15 cc. of $\frac{N}{100}$ solution (0.286 per cent) of the sodium salt were used there were no symptoms whatever. At autopsy it was found that the peritoneal surfaces were glistening and covered between the coils of intestine by a small amount of blood stained serous fluid. Beneath the abdominal wound and adherent to the parietes there was a mass of blood stained fibrin. The omentum showed minute haemorrhages and there was a similar fibrinous mass adherent to it. There were scattered minute subserous haemorrhages over the intestines and the parietal peritoneum in the pelvic region. With stronger solutions the irritating action of the substance is much more marked and may prove fatal.

Iodoxybenzoic acid: On account of its markedly acid properties, free iodoxybenzoic acid is more irritating than free iodosobenzoic acid, but the sodium salt of iodoxybenzoic acid is in comparison to sodium iodosobenzoate a bland substance. One cubic centimeter of $\frac{N}{30}$ sodium iodoxybenzoate was injected subcutaneously in a rabbit and 8 cc. of $\frac{N}{30}$ solution were placed in the peritoneal cavity. There were no symptoms. Forty-eight hours later the rabbit was killed by a blow on the neck. The site of the subcutaneous injection was dry and showed no sign of haemorrhage or filtration. The abdominal wound was clean and showed a few minute subcutaneous haemorrhages in the immediate vicinity. There was a small collection of fibrin on the peritoneal surface of the wound and one or two flakes of fibrin on the surface of the intestine. The peritoneal surfaces were moist and glistening and there was no excess of fluid in the peritoneal cavity, which was perfectly normal except for one minute haemorrhage in the omentum. The lungs, heart and all the organs were perfectly normal.

We may summarize the local action of the three acids and their sodium salts as follows: Iodbenzoic acid is slightly irritating in consequence of its acid character. Free iodosobenzoic acid when pure is a very bland substance. When dusted into the eye or when taken into the stomach it produces no reaction. Iodoxybenzoic acid is quite irritating in consequence of its acid properties. Sodium iodbenzoate and iodoxybenzoate are quite bland. Sodium iodosobenzoate is exceedingly irritating especially to the subcutaneous tissue and the serous surfaces.

A comparison of taste of iodbenzoic, iodosobenzoic and iodoxybenzoic, as well as that of their sodium salts is quite interesting. Iodbenzoic acid has a very faint sour taste when the dry powder is placed on the tongue. Iodosobenzoic acid has the peculiar taste of hydrogen peroxide. In larger quantities it causes a peculiar peppery sensation.

Iodoxybenzoic acid has an intensely sour taste as one should expect from its strongly marked acid properties. It also has a metallic taste and causes the peppery sensation noted in the case of iodosobenzoic acid. In comparing the tastes of the sodium salts of the three acids $\frac{N}{100}$ solutions were employed. Several persons tasted the solutions for us without having any knowledge of what was expected of them. Of the three salts that of iodosobenzoic acid has by far the strongest taste and its taste is almost identical with that of hydrogen peroxide, so much so in fact, that one is surprised to find that the saliva is not frothy as occurs when hydrogen peroxide is taken into the mouth. Pure sodium iodbenzoate is practically tasteless in $\frac{N}{100}$ solution. Sodium iodoxybenzoate also tastes very much like hydrogen peroxide solutions but the taste is not nearly as strong as that of sodium iodosobenzoate solutions of corresponding strength.

THE PHYSIOLOGICAL AVAILABILITY OF THE OXYGEN COMBINED WITH THE IODINE IN IODOSOBENZOIC AND IODOXYBENZOIC ACIDS

As already pointed out the oxygen combined with the iodine in these compounds is very active. It is capable of quantitatively oxidizing hydriodic acid with the liberation of iodine instantly at room temperature. Nevertheless the ordinary chemical activity of the oxygen in any compound does not necessarily mean that such oxygen will be available physiologically. The sodium salts of both acids immediately oxidize haemoglobin to oxyhaemoglobin.⁹ A dilute solution of oxyhaemoglobin obtained by

⁹ It should be pointed out that on treating the sodium salts of iodoso and iodoxybenzoic acids with blood there is no liberation of gaseous oxygen as in the case of hydrogen peroxide.

laking blood with distilled water was treated with Stokes reagent until the two absorption bands of oxyhaemoglobin fused into the single band of haemoglobin and the color of the blood indicated the change from oxyhaemoglobin to haemoglobin. On adding the $\frac{N}{30}$ solution of the sodium salt of either iodoso- or iodoxybenzoic acid the two bands of oxyhaemoglobin immediately appeared and the color changed back to the bright red of an oxyhaemoglobin solution.

This very simple experiment proved that the oxygen which these substances contain is available physiologically through the intermediation of haemoglobin, but we were also anxious to determine whether or not the tissues themselves might not be able to utilize the oxygen combined with the iodine in these substances in much the same manner that they utilize the molecular oxygen, which in the higher animals is derived from the oxyhaemoglobin. So far as we know no attempt has been made to supply oxygen to the tissues in any other form than as molecular oxygen.¹⁰

In order to form some idea of the direct physiological availability of the active oxygen contained in our substances we have made use of the various oxidase and peroxidase reactions.

Neither the free acids nor the sodium salts are capable of oxidizing either guaiacum or aloin in the presence or absence of blood or tissue extracts so that as far as these substances are conserved our results are purely negative. Positive results were obtained, however, when we employed the sodium salt of phenolphthalin, the oxidase and peroxidase reagent introduced by Kastle and Shedd.¹¹ This substance was used by Kastle¹² in his extensive studies on the peroxidase of blood. Sodium iodosobenzoate is incapable of oxidizing phenolphthalin to phenolphthalein under

¹⁰ By this statement we mean any modern attempt by experimental methods. The old idea that the chlorates given by mouth supply the body with oxygen is only of historical interest as showing how completely the application of ordinary test tube chemistry fails when applied to living things without experimental basis. This is perhaps more true in oxidative reactions than in reactions of any other type.

¹¹ Amer. Chem. Jour., 26, 526 (1901).

¹² "Variations in the peroxidase activity of the blood in health and disease." Washington, D. C., Hygienic Laboratory Bulletin, No. 31, 1906.

the conditions of our experiments, but in the presence of diluted blood it effects this oxidation, thus indicating that the oxygen contained in sodium iodosobenzoate is capable of being activated by the peroxidase of the blood in this reaction and supporting the view that the active oxygen which it contains is probably capable of being utilized directly by the tissue in certain oxidations. The following may be given a typical experiment: Mixtures of diluted human blood (diluted approximately 1 : 1000) $\frac{N}{100}$ sodium phenolphthalin, $\frac{N}{100}$ sodium iodosobenzoate and water were placed in test tubes. The total volume was in each case made up to 7 cc.

- | | |
|---|--|
| (1) 0.2 cc. phenolphthalin
6.8 cc. water | (4) 0.2 cc. phenolphthalin
0.2 cc. iodosobenzoate
0.2 cc. diluted blood
6.4 cc. water |
| (2) 0.2 cc. phenolphthalin
0.2 cc. diluted blood
6.6 cc. water | (5) 0.2 cc. diluted blood
6.8 cc. water |
| (3) 0.2 cc. phenolphthalin
0.2 cc. iodosobenzoate
6.6 cc. water | (6) 0.2 cc. diluted blood
0.2 cc. sodium iodosobenzoate
6.6 cc. water. |

After standing sixteen hours at room temperature (20°) 1 cc. $\frac{N}{10}$ sodium hydroxide was added to each tube with the following results:

- (5) Colorless.
(6) Yellowish.

(1), (2) and (3) were of about the same tint, namely, very faintly pink.

(4) Much deeper pink, and when compared with (1), (2) and (3) in the Duboscq-Soleil colorimeter it was found to be just three times as pink as (1), (2) and (3).

A few experiments performed with sodium iodoxybenzoate did not indicate that the oxygen of this substance is capable of furnishing oxygen in this reaction. The results are not conclusive, however, and it is our intention to return to this problem as soon as the opportunity presents itself. The problem is one of considerable interest in itself, but we do not wish to attach too much importance to it so far as the physiological availability of the oxy-

gen is concerned. It is perfectly conceivable that a substance which cannot furnish oxygen for this reaction may still be utilized as an oxidizing agent by cells for physiological oxidations and the reverse of this is also probably true. The study justifies itself because it is the only evidence which we can obtain in test-tube experiments of the probably physiological availability of the oxygen in any given compound.

The effect of sodium iodosobenzoate and iodoxybenzoate on the blood: In this part of the work we have been assisted by Dr. C. H. Bunting, who made all of the blood counts. He counted the leucocytes, the erythrocytes, and, in some cases, also the platelets. We desire to express our thanks to him for his valuable help. We have made the haemoglobin determinations ourselves using the Miescher apparatus.

The drugs were injected by placing a canula into the external jugular vein of rabbits under local anaesthesia and as nearly aseptically as possible. The insertion of the canula was rendered necessary because it was desired to give the drugs slowly and in considerable volume, conditions which preclude ear vein injection.

Experiment 2. Rabbit, male, 1790 grams, 15 c.c. of $\frac{N}{30}$ sodium iodosobenzoate were injected during a period of twenty-nine minutes

	HAEMOGLOBIN	ERYTHROCYTES	LEUCOCYTES	COAGULATION TIME
	<i>per cent</i>			
Five hours before injection..	93	6,384,000	9,000	3 minutes
Injected 15 cc. $\frac{N}{30}$ sodium iodosobenzoate.....				
Twenty hours after injection	73	6,016,000	19,000	4 minutes
Forty-three hours after injection.....		5,600,000	11,000	3 minutes 30 seconds
Sixty-eight hours after injection.....	72	5,360,000	11,000	3 minutes 15 seconds

Professor Bunting also made differential counts, the results of which were as follows:

	NOVEMBER 3 (BEFORE INJECTION)		NOVEMBER 4		NOVEMBER 5		NOVEMBER 6	
	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Polymorphonuclears.....	2160	24.0	11362	59.8	4422	40.2	4246	38.6
Eosinophiles.....	180	2.0	76	0.4	176	1.6	154	1.4
Basophiles.....	288	3.2	380	2.0	330	3.0	176	1.6
Small mononuclears.....	5922	65.8	6422	33.8	4818	43.5	5390	49.0
Large mononuclears.....	450	5.0	760	4.0	1254	11.4	1034	9.4
Totals.....	9000		19000		11000		11000	

The large leucocytosis noted in this rabbit is thus shown to be due almost entirely to the increase in the polymorphonuclear leucocytes which after twenty-four hours were found to be increased more than five times. On November 5, forty-four nucleated red cells were found indicating blood regeneration.

With this large amount of sodium iodosobenzoate, corresponding to 0.132 gram of free iodosobenzoic acid, there was some blood destruction although this was not severe.

At the completion of the experiment the rabbit was killed by a blow on the neck and an autopsy was done. The only noteworthy finding at autopsy was the congestion of both lungs at the base. In the right lung there was a triangular haemorrhagic area of considerable size. The condition of the lungs was probably sufficient to account very largely for the degree of anaemia noted.

Experiment 3. Rabbit, albino, female, 1500 grams. In a period of 32 minutes 15 cc. $\frac{N}{30}$ sodium iodosobenzoate were injected into the external jugular vein

	HAEMO- GLOBIN	LEUCOCYTES	ERYTHROCYTES	COAGULATION TIME
	<i>per cent</i>			
Before injection.....	86	10,000	6,224,000	2 to 2 $\frac{1}{4}$ minutes
Fifteen cc. $\frac{N}{30}$ sodium iodo- sobenzoate injected.....				
Twenty-two hours after in- jection		13,500	6,032,000	2 minutes
Seventy hours after injection		12,500	6,480,000	1 $\frac{1}{2}$ to 2 minutes
One hundred and twenty-five hours after injection.....	85			

In this case practically no blood destruction was noted, although the same dose was used as in Experiment 2 and the rabbit was smaller. The leucocytosis in this case was much less marked than in the previous one.

In Experiment 1 in which a male rabbit of 1800 grams was used the platelet count fell from 670,000 to 470,000 within $3\frac{1}{2}$ hours after injecting 30 cc. of a $\frac{N}{30}$ solution of the iodosobenzoate. The ratio of platelets to red cells fell from 1:7.5 to 1:10 during the interval. There was no oedema of the lungs.

Experiment 5. Rabbit, female, 2100 grams

	ERYTHROCYTES	LEUCOCYTES
Before injection.....	5,400,000	17,000
Injected 10 cc. $\frac{N}{33\frac{1}{2}}$ sodium iodoxybenzoate		
Nineteen hours after injection.....	5,000,000	25,000
Forty-two hours after injection.....	5,416,000	17,500

No nucleated reds appeared in the circulating blood. The rabbit remained entirely well. Many other experiments gave similar results. The conclusions to be drawn regarding the effect of the substances on the blood when injected intravenously are as follow:

1. Both sodium iodosobenzoate and iodoxybenzoate usually cause a more or less marked leucocytosis affecting especially the polymorphonuclear leucocytes.

2. Iodosobenzoate, when injected in quantities of 15 cc. $\frac{N}{30}$ solution in rabbits causes a certain decrease in the red cells in the haemoglobin. In many cases the destruction is entirely insignificant, and in no case was it great.

3. After iodosobenzoate in large doses rabbits often show a haemorrhagic condition of the lungs. There may be simply a very marked congestion or in some cases haemorrhage into the lung tissue may be noted.

As further evidence of the extreme degree of congestion of the lungs, which may occur in some cases following iodosobenzoate, we have frequently noted in cats, that just before death there escapes from the trachea very large amounts of a blood stained

frothy fluid. In other cases neither oedema nor congestion of the lungs was found at autopsy.

THE EFFECT OF SODIUM IODBENZOATE, IODOSOBENZOATE, AND
IODOXYBENZOATE ON THE CIRCULATION AND RESPIRATION

In order to elicit the effect of these substances on the circulation and respiration it is necessary that they be given intravenously. The reason for this is easily seen from what follows. The activity of both iodoso- and iodoxybenzoate is due to the presence of active oxygen—the reduction product is quite inactive in corresponding doses. In their passage into the blood stream either from the gastro-intestinal tract or from the subcutaneous tissues, much or all of this active oxygen is lost. Hence in discussing the action of the substances on the circulation and respiration we refer in all cases to experiments in which the salts are given intravenously.

Action on the circulation

As previously pointed out, sodium iodbenzoate does not contain active oxygen and it therefore furnishes a valuable control in studies of iodoso- and iodoxybenzoate. It will be remembered that iodosobenzoic acid is an exceedingly weak acid, whereas iodoxybenzoate acid is quite strong. Furthermore these two substances behave quite differently in certain chemical reactions. Thus Hartmann and Meyer found that iodosobenzoic acid on treatment with alkalis yields salicylate, whereas iodoxybenzoic acid, although richer in oxygen, yields the salts of benzoic and iodic acids, but no salicylate. Hence iodoso- and iodoxybenzoates differ widely from one another in their general chemical properties. They have one chain of properties in common, namely, those dependent on the presence of chemically very active oxygen. On the other hand, iodbenzoic acid differs from the other substances principally in that it contains no active oxygen. Hence any pharmacological property possessed by both iodoso- and iodoxybenzoates and not possessed by iodbenzoate may fairly be attributed to the active oxygen which the former substances contain.

Sodium iodbenzoate: With the vagi intact the injection of 5 cc. of $\frac{N}{20}$ sodium iodbenzoate in the cat or rabbit usually causes a rise of arterial blood pressure, which, though not large, lasts for some minutes (see fig. 7). The rise is usually 8 to 10 mm. of mercury, although a rise of as much as 20 mm. was noted in some cases. Sometimes there is no increase in the blood pressure but the point of interest is that it never causes a fall of blood pressure—at least it has never occurred in our experiments. At no time during its action does it cause any change in the pulse rate. The very slight decrease in the pulse rate in one experiment was probably secondary to the considerable rise of blood pressure. With the blood pressure high as the result of vagal section, iodbenzoate does not cause a rise of blood pressure. The harmless character of the salt when injected intravenously stands out in marked contrast to the other salts. In an experiment on a cat 25 cc. of the $\frac{N}{20}$ solution were injected before cutting the vagi, and the same amount after cutting the vagi, corresponding in all to 0.64 gram of free iodbenzoic acid and at the end of the experiment the blood pressure was 180 mm. and the pulse rate 238. At the beginning of the experiment, with the vagi intact, the blood pressure was 149 mm. and the pulse rate 208. This indicates how bland the salt is when injected intravenously. We have not determined the factors in the rise of blood pressure, but certain facts would indicate that it is probably largely due to increased cardiac output.

Sodium iodosobenzoate: We studied the action of this substance in a large number of experiments using cats, rabbits and dogs. The action of the salt on the circulation is essentially the same in all three animals. We shall describe the typical action of the salt first and then deal with the exceptions. The dose required to elicit the typical action varies somewhat in different animals, even of the same species.

In the cat the first indication of the typical effect of the salt on the circulation is seen when 1 cc. of the $\frac{N}{20}$ solution (corresponding to 0.0132 gram of the free acid,) is injected intravenously. It may be necessary to repeat this dose in order to elicit the action

of the salt to even a slight extent. The full activity of the salt is seen after the injection of 5 cc. $\frac{N}{20}$ solution (0.066 gram of the free acid). In this case there is at first a sudden fall of blood pressure of often 40 mm. of mercury within 10 to 20 seconds following the injection. The blood pressure then continues to fall, but more gradually for the next minute or more, during which period it may show a further fall of 10 to 20 mm. of mercury. The blood pressure recovers very slowly. There is no change in the pulse rate at any time. In order to determine the factors in this fall of blood pressure we have studied the effect of the salt: (1) On the volume changes of the heart using a cardio-plethysmograph connected with a piston recorder. (2) On the volume of a loop of the intestines before and after cutting the splanchnics. (3) On the rate of outflow from the mesenteric vein on perfusing the isolated intestines using the Wiggers outflow recorder.

1. *Studies with the cardioplethysmograph.* The injection of 3 cc. $\frac{N}{20}$ sodium iodosobenzoate into the saphenous vein (cat) caused a marked fall of blood pressure. During the time that the principal fall of blood pressure occurred there was practically no change in the rate or amplitude of the heart beat. After about one minute following the injection, and while the blood pressure was still falling, there was noted a loss of tone affecting systole much more than diastole and resulting, at the height of the effect, in a decrease of about 15 per cent in the amplitude of the beat. The heart rate dropped from 36 to 34 in ten seconds. This effect was transitory. Following this the amplitude gradually increased until it was about 15 per cent above the normal and the rate fell to 31 in ten seconds. It required from $2\frac{1}{2}$ to 3 minutes for the heart to regain its previous tone. A second injection of 3 cc. $\frac{N}{20}$ solution again caused a marked fall of blood pressure. Here also, after the principal fall of blood pressure had occurred, the heart became dilated, the amplitude decreased markedly and the beat became irregular. The dilatation became extreme four minutes after the injection and remained so for about two minutes, when it slowly recovered and the heart beat became almost normal again. During the period of cardiac dilatation the blood pressure fell to 16 mm. Figs. 1 and 2 reproduce tracings from

which the above description was taken. The result was confirmed by other experiments.

It is impossible to say whether or not the cardiac dilatation and the decreased cardiac output is the cause of the gradual long continued fall of blood pressure which occurs after the first rather precipitous fall. It may be that the cardiac dilatation is the result, rather than the cause, of the fall of arterial blood pressure and the increase in the venous pressure resulting from the dilatation of the peripheral vessels. The improvement in the cardiac condition and the rise of blood pressure occur simultaneously. The fact that most of the fall of blood pressure occurs before the efficiency of the heart is materially impaired would indicate that the decreased efficiency and dilatation of the heart are the result of the fall in the arterial, and the rise in the venous pressure. The conclusion from the work is that the fall of blood pressure under sodium iodosobenzoate is certainly not entirely cardiac in origin.

2. The effect of the salt on the volume of a loop of the small intestine before and after cutting the splanchnics, showed clearly that the fall of blood pressure is due to a dilatation of the blood vessels which is dependent on their connection with the nervous system. Before severing the splanchnic nerves the iodosobenzoate caused the volume of the loop of intestine to increase while the blood pressure was falling, whereas, after cutting the splanchnics, the volume of the loop ran parallel to the blood pressure (figs. 3 and 4).

3. In order to preclude further any direct effect on the vessel wall, the intestines were perfused through the mesenteric artery and the rate of outflow from the vein recorded. All nervous connection was severed. Locke's solution without bicarbonate was used as the perfusion fluid. The iodosobenzoate was made up with Locke's solution to a $\frac{N}{1000}$ or $\frac{N}{1700}$ solution. In neither case did the salt have the slightest effect on the rate of outflow.

The initial fall of blood pressure is therefore due to depression of the vaso-motor center.

Sometimes iodosobenzoate causes a rise of blood pressure. The most marked case was in an experiment in which a cat was

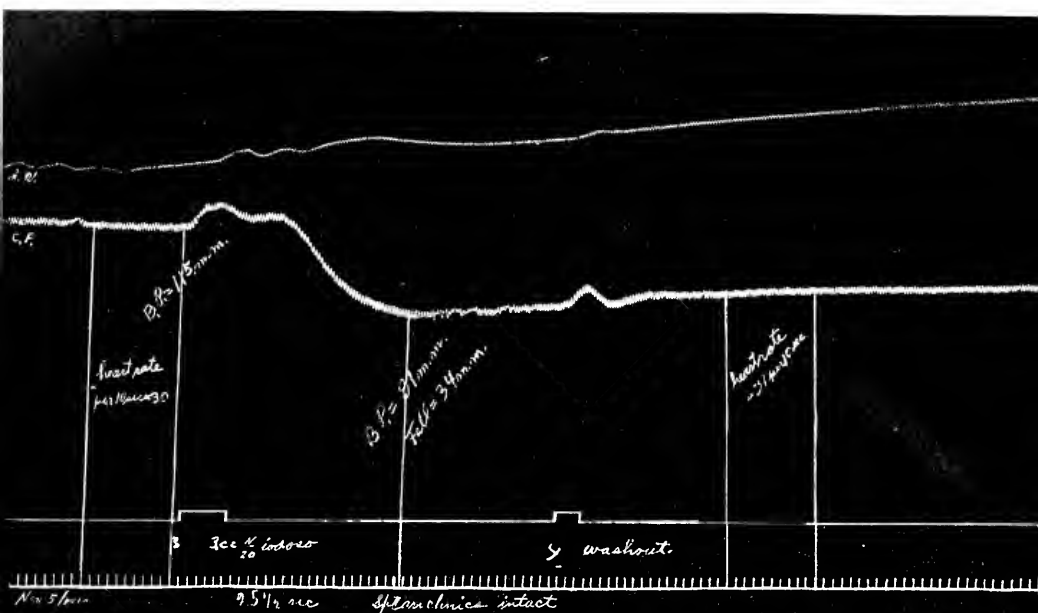


Fig. 3. Cat, *a*, Volume of loop of intestine, piston recorder. *b*, Carotid pressure. *c*, Splanchnic and zero pressure. *d*, Time in seconds. At 3.3 cc. $\frac{N}{20}$ iodosobenzoate injected into femoral Splanchnics intact. Read left to right. (Reduced to $\frac{2}{3}$.)

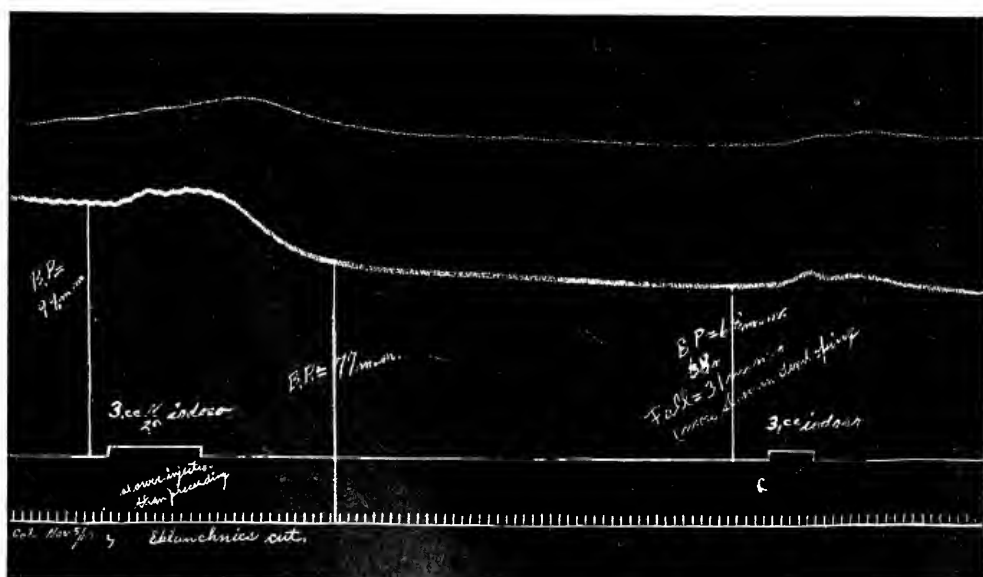


Fig. 4. (From same animal as Fig. 3.) The splanchnics had been cut. At 7.3 cc. $\frac{N}{20}$ iodosobenzoate were injected into femoral vein.

used. In this case the first injection of 5 cc. $\frac{N}{20}$ iodosobenzoate caused tremendous stimulation although the animal was deeply anaesthetized with ether. The struggling interfered with the recording apparatus so that the height of blood pressure was not recorded. However, when the struggling ceased the blood pressure on the return toward normal was 182 mm. compared with a previous normal pressure of 162 mm. The pulse rate at this time was 256, the normal being 194. A second injection of the same dose in this animal caused a rise of blood pressure from the normal of 156 mm. to 220 mm. of mercury, the pulse rate increasing from 176 to 216. Subsequent injections produced the typical fall of blood pressure. In a few cases the iodosobenzoate caused first a fall and later a considerable rise in the blood pressure. Our cardioplethysmographic records indicate that the secondary rise is probably due to increased cardiac output. When very small quantities of iodosobenzoate are used (1 cc. $\frac{N}{1000}$ corresponding to 0.00264 gram of the free acid), an insignificant rise of blood pressure may be noted. The cause of the rise of blood pressure which is sometimes seen with the iodosobenzoate has not been determined. In the cases associated with struggling it is probably due to the muscular contraction. In other cases where there is no struggling it is in all probability cardiac in origin. We have always found that at certain stages of the action the cardiac output is increased, and when this is unusually large and not sufficiently antagonized by depression of the vaso-motor center, the net result may be a rise of the arterial blood pressure.

Sodium iodoxybenzoate: The action of this substance on the circulation is identical in its general characteristics with that of the iodosobenzoate. It is simply less powerful than the iodosobenzoate. The fall in the blood pressure in the typical cases is usually less marked and it causes a rise of blood pressure in a larger percentage of the cases than does the iodosobenzoate. It is not so depressing on the heart and an increase in the cardiac output is the rule. Again it requires more of this salt to permanently injure the heart. For these reasons the fall of blood pressure

under iodoxybenzoate, even though it caused as large an initial fall is more rapidly recovered from than in the case of the iodosobenzoate. A typical experiment may be given. Five cubic centimeters of a $\frac{N}{20}$ solution were injected into the saphenous vein of a cat. The blood pressure fell within eighteen seconds from 120 to 70 mm. The blood pressure did not continue to fall from this point as in the case of iodosobenzoate but it required about ten minutes to return to normal. Cardioplethysmographic tracings showed that the fall of blood pressure is not due to any cardiac effect of the drug. Perfusion of the isolated intestines recording the rate of outflow proved that the salt does not act directly on the vessel wall and here again we conclude that the salt lowers the blood pressure by depressing the vaso-motor center. We believe that the rise of blood pressure not infrequently seen, is due mainly to increased cardiac output, although we have not excluded the possibility that under certain conditions the salt may stimulate the vaso-motor center.

The respiration

By far the most interesting and significant action of these substances is on the respiration. As in the case of the circulation the salts must be injected intravenously in order to elicit their characteristic effect.

The respirations were recorded by several methods and frequently two methods were used in the same experiment. In most cases the respiration was recorded by a tambour connected with the trachea tube, and in addition in the experiments with rabbits, Head's method of recording the respiration from the slip of the diaphragm was employed. In many of the experiments with cats the respiration was also recorded directly from the diaphragm by means of a thread connecting the diaphragm with a receiving tambour, which was in turn connected with a recording tambour, using air transmission.

Sodium iodbenzoate: In ordinary doses of 5 cc. $\frac{N}{20}$ solution or less this substance does not affect the rate or amplitude of the respiration in the cat, dog or rabbit. Hence in regard to its action on both the respiration and the circulation this substance is bland.

Sodium iodosobenzoate and sodium iodoxybenzoate: These substances have practically identical effects on the respiration, so that they may be considered together. Their characteristic effect on the respiration is the production of apnoea.¹³ The respiration always stops in repose, passive expiration. Apnoea may be produced by the salts either with the vagi intact or cut. The latter condition, however, favors the production of apnoea. Extremely long periods of apnoea were obtained with the vagi cut. The interval between the injection and the production of apnoea, as well as the duration of the apnoea, depends upon the dose and apparently upon other factors of which we are entirely ignorant. In some of our experiments, apnoea has occurred immediately at the completion of the injection of the salt—the injection having lasted 10 to 12 seconds. In one case the apnoea began seven seconds after an injection requiring two seconds. In other cases the interval was much longer, varying between eight seconds and four minutes in our series. One of the most interesting cases of a long interval between the injection and the production of apnoea was the following, which also illustrates the fact that it is easier to produce apnoea with the salts when the vagi are cut than when they are intact.

Experiment 14. A cat (3400 grams) received four injections of $\frac{N}{20}$ sodium iodoxybenzoate of 2.5, 2.5, 5 and 5 cc. respectively within a period of $8\frac{1}{2}$ minutes without more than a slight depression of the respiration. Seventeen minutes later the vagi were cut and about three minutes later there occurred an apnoea which lasted ninety-seven seconds. This apnoea began twenty minutes after the last injection.

The duration of apnoea in our series of twenty-five experiments

¹³ The propriety of using the term "apnoea" in this connection will probably be questioned. The term really means a temporary cessation of the respiration. Miescher distinguished two forms of apnoea designating that form due to changes in the gaseous composition of the blood as "apnoea vera" and that due to the stimulation of the vagus and the sensory nerves of the larynx and nasal mucosa as, "apnoea spuria." The same chemical changes in the cells of the center may condition both forms of apnoea. We here use the term apnoea because we believe the cause of the respiratory stoppage to be essentially the same as in apnoea vera. The reasons for this conclusion will be discussed in a later paper by one of us.

varied from a few seconds up to one hundred and fifty-one seconds. Many of the records show an apnoea of from 1 to 2 minutes. In some experiments apnoea did not occur. By starting with small doses it can be obtained in the cat and rabbit almost invariably. Apnoea can also be produced in the dog, but large amounts of the substance may be required, and therefore we have not used dogs in many experiments. To illustrate how much of the salts dogs will stand, we may cite Experiment 5, in which a dog of 6.5 kilos received 247 cc. of a $\frac{N}{26.4}$ solution of sodium iodosobenzoate within a period of $1\frac{1}{2}$ hours. This caused a respiratory pause of only four seconds. The respiratory rate was decreased. This quantity of the salt did not kill the animal. In Experiment 4 a dog of 10.1 kilos received 153 cc. of a $\frac{N}{26.4}$ and 25 cc. of a $\frac{N}{13.2}$ solution of sodium iodosobenzoate. There occurred a respiratory pause of thirty-seven seconds. Death in this case was due to the salt. The heart continued to beat forty-three seconds after the respiration stopped.

Death under these substances is due almost invariably to failure of the respiration, the heart continuing to beat from thirty seconds to five minutes after the respiration ceases.

The production of apnoea by the salts is entirely independent of blood pressure changes. They were obtained with a rise of blood pressure in some cases and with a fall of blood pressure in others. Sometimes the injection of the salts is not followed by apnoea, but the depression of the respiration is merely indicated by a decrease in the rate and depth of the respiration.

So far we have only spoken of respiratory depression, but we also have abundant evidence of stimulation of the respiration by the salts. The primary effect is occasionally an increase in the rate and depth of the respiration. This usually occurs after large doses and threshold doses seldom cause dyspnoea.¹⁴

Frequently the injection of a good sized dose, such as 5 cc. $\frac{N}{20}$ solution, causes for a very brief period a decrease in the rate and depth of the respiration immediately following the injection and lasting but a few seconds to be replaced by extreme dyspnoea.

¹⁴ There is great variation in the dose required to produce the same effect in different animals.

A probable explanation of this apparently paradoxical result will be considered in a later paper by one of us, dealing with a theory of the respiration. A few typical examples of the effect of the salts on the respiration may be given.

In cats and rabbits the threshold dose usually required to produce an effect on the respiration, may be placed at 1 or 2 cc. $\frac{N}{100}$ solution, which corresponds to 0.00264 to 0.00528 gram of

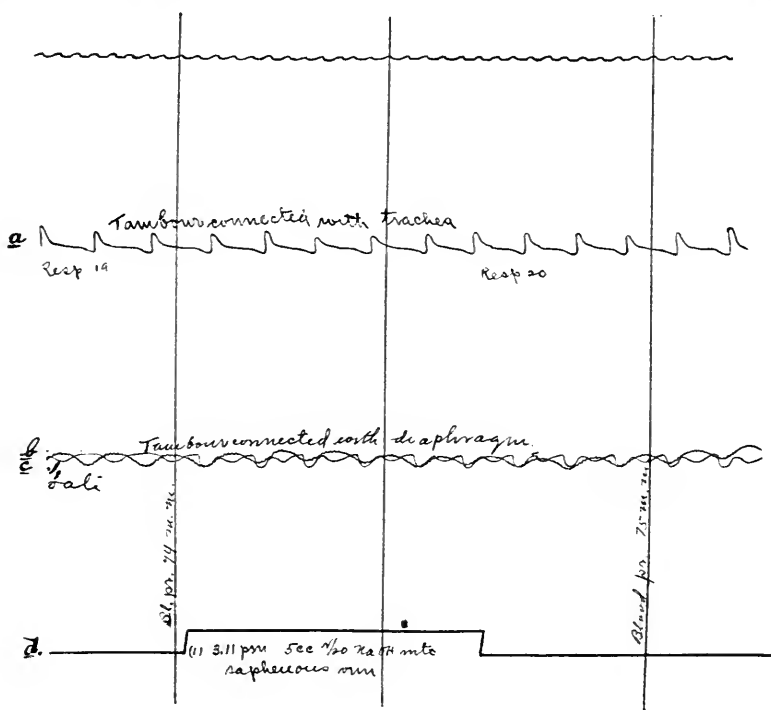


Fig. 5. At 3.11, 5 cc. $\frac{N}{100}$ NaOH injected into saphenous vein. Tracings same as in Fig. 6.

the free acid. In some animals 1 cc. or more of $\frac{N}{100}$ solution may be required to elicit the effect. In a cat of 4.3 kilos (Experiment 24) the injection of 1 cc. $\frac{N}{100}$ solution of iodosobenzoate was followed ten seconds later by a respiratory pause of five seconds, and thirty-five seconds later another pause of five seconds occurred. The respiratory rate was decreased from 32 to 28 per minute and remained below normal until the next injection was made.

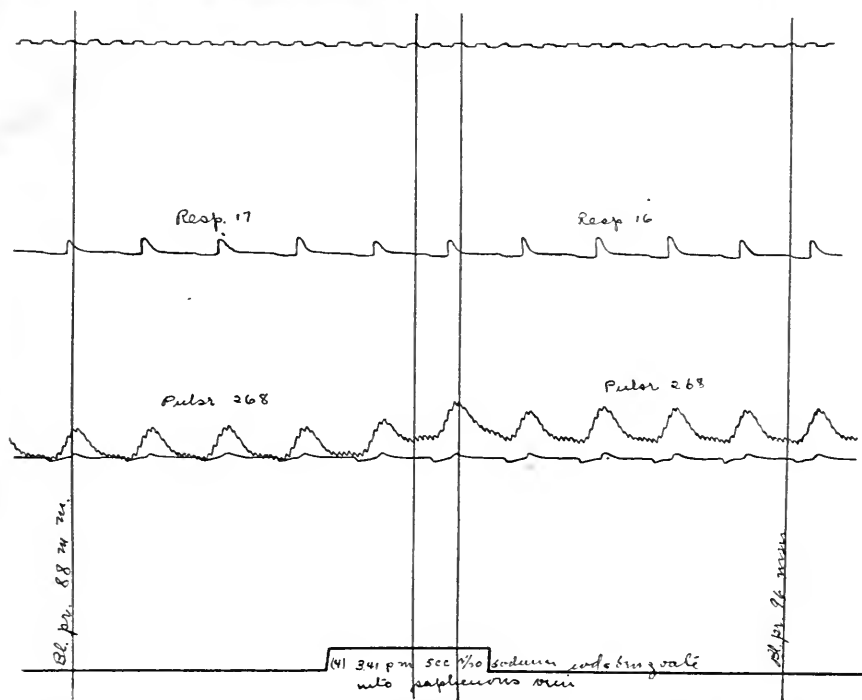
The amplitude of the respiration was very slightly increased. Dyspnoea did not follow this injection. The effect was purely a depression of the respiration. Two minutes after this injection a second injection of 2 cc. $\frac{N}{100}$ iodosobenzoate was made. Seven seconds later there occurred an apnoea of $14\frac{1}{2}$ seconds duration. This dose was followed by a rise of blood pressure (114 to 120 mm.) most of which occurred during the apnoea. Following the apnoea there was a very slight increase in the rate and depth of the respiration. There was no dyspnoea.

A cat of 2400 grams (Experiment 13), in which the vagi had been cut received at 4:13 p.m. 5 cc. $\frac{N}{20}$ iodosobenzoate. There occurred several long pauses in the respiration immediately following the injection. After it became more regular the rate was reduced from 20, the normal, to 16 per minute. At 4:16, 3 cc. $\frac{N}{20}$ iodosobenzoate were injected. This further reduced the respiratory rate to 13. At 4:17 $\frac{1}{2}$, 5 cc. $\frac{N}{20}$ iodosobenzoate were injected. The respiratory rate, which had returned to 20 was immediately reduced to 14, and gradually diminished in size until after seventy-four seconds an apnoeic period of twenty seconds occurred. Respiration was then resumed, at first of fair depth but gradually diminishing in rate and size. After 11 respirations within fifty-six seconds a period of apnoea lasting twenty-five seconds occurred, followed by a single shallow respiration and then apnoea of eighty-six seconds. The blood pressure remained above 50 mm. until the last few seconds when it dropped a few millimeters. Following the long apnoea, there gradually developed most extreme dyspnoea, lasting about two minutes when the respiration finally ceased, the heart continuing to beat one hundred and sixty-four seconds after the last respiration. This experiment illustrates fatal apnoea under the salt, and closely resembles the fatal apnoea described by Henderson¹⁵ but produced by apparently entirely different means. We shall try to show later that the mechanism is probably essentially the same. It is very difficult to determine whether iodosobenzoate or iodoxybenzoate acts the more powerfully on the respiration, as it is difficult to get comparable experiments in the same animal since the animal seldom responds to the same extent

¹⁵ Amer. Jour. of Physiol, 25, 310 (1910).

to successive doses. In some animals an increased susceptibility and in other animals a decreased susceptibility to the second dose is noted. The effect of iodosobenzoate in depressing the circulation so much more than iodoxybenzoate, tends to enhance its effect upon the respiration.

The great difference noted between the effects on the respiratory and vaso-motor centers of iodbenzoate on the one hand, and



g. 8. From same experiment as Figs. 5, 6 and 7. At 3.41 P.M. 5.cc. $\frac{N}{20}$ sodium iodbenzoate injected into saphenous vein.

iodosobenzoate and iodoxybenzoate on the other, must be attributed to the active oxygen which the latter substances contain. As previously pointed out, the latter substances differ from iodbenzoic acid in the possession of this active oxygen. Iodosobenzoate and iodoxybenzoate have the same effect on the respiratory center and yet they differ quite markedly from one another chemically. The common pharmacological activity must be due to

their common chemical potentiality, namely, the presence of active oxygen in available form. In the following experiment (No. 17) it would appear that the iodoxybenzoate is the more powerful in its effect upon the respiration. In this experiment 5 cc. $\frac{N}{20}$ sodium hydroxide was first given as a control to exclude any possible alkali effect, then 5 cc. $\frac{N}{20}$ sodium iodoxybenzoate were given at 3:17 $\frac{1}{4}$ p.m. At 3:33 p.m. 5 cc. $\frac{N}{20}$ sodium iodosobenzoate were injected and at 3:41 5 cc. $\frac{N}{20}$ sodium iodbenzoate. The animal used was a female cat weighing 2100 grams. The vagi were intact. The record speaks for itself and requires no comment. (Fig. 5, 6, 7 and 8.) We shall consider certain other substances which cause temporary cessation of the respiration in the communication following this one.

SUMMARY

1. Improved methods for making and purifying iodbenzoic, iodosobenzoic, and iodoxybenzoic acids are described.

2. The local action of the free acids and their sodium salts are described. The results show that of the sodium salts, those of iodbenzoic and iodoxybenzoic acids are mild, while that of iodosobenzoic acid is extremely irritating when injected subcutaneously or intraperitoneally.

3. It is shown that sodium iodosobenzoate and iodoxybenzoate, in dilute solution, have a peculiar metallic taste very much like that of hydrogen peroxide and it is suggested that the active oxygen confers upon all of these compounds their peculiar taste.

4. Solutions of sodium iodosobenzoate and iodoxybenzoate immediately oxidize haemoglobin to oxyhaemoglobin.

5. The peroxidase of the blood enables sodium iodosobenzoate to oxidize phenolphthalin to phenolphthalein; in other words, it can furnish oxygen for this peroxidase reaction in the same manner that hydrogen peroxide does.

6. Sodium iodosobenzoate and sodium iodoxybenzoate, when injected intravenously, do not exercise a destructive action on the blood to any extent. Leucocytosis of a moderate grade may be noted.

7. In order to elicit their characteristic effects on the circulation and respiration the salts must be injected intravenously.

8. On the Circulation: *Sodium iodbenzoate* is but very slightly active, causing a rise of arterial blood pressure. The mechanism by which this is brought about was not determined.

Sodium iodosobenzoate usually causes a marked and long continued fall of blood pressure. During the time that the blood pressure shows the greater part of the fall, the heart rate and amplitude are hardly affected. The salt causes dilatation of a loop of intestine when the splanchnic nerves are intact, but not after they are cut. It does not affect the caliber of blood vessels by peripheral action. Hence it depresses the vaso-motor center. At certain stages the salt increases the cardiac output and we believe this to be the mechanism involved in the rise of blood pressure occasionally noted.

Sodium iodoxybenzoate acts similarly to the iodosobenzoate on the circulation, but its action is not so marked. It causes a rise of blood pressure in a larger per cent of the cases than iodosobenzoate and is less depressing on the heart. The usual fall of blood pressure is due to depression of the vaso-motor center.

9. On the Respiration: *Sodium iodbenzoate* contains no active oxygen and in the doses we employed it does not affect the rate or depth of the respiration. *Sodium iodosobenzoate* and *sodium iodoxybenzoate* have about the same effect on the respiration. They both cause cessation of the respiration in repose, in dogs, cats and rabbits. The best results are obtained with cats. The period of apnoea usually begins within a few seconds after starting the injection and lasts from a few seconds up to 2 or 3 minutes and is terminated by spontaneous return of the respiration. Apnoea may come on several minutes after the last injection. Apnoea is more easily produced by the drugs after section of the vagi, but this is not essential. Apnoea may occur with a rising or a falling blood pressure and is independent of it. During the apnoea there is usually a fall of blood pressure, while the pulse rate remains normal.

10. Of the medullary centers, the respiratory center is the most sensitive to *sodium iodosobenzoate* and *iodoxybenzoate*.

The vaso-motor center is next and the cardio-inhibitory center is not affected by them.

11. It is pointed out that the remarkable effect of sodium iodosobenzoate and iodoxybenzoate on the respiration and also their effect on the vaso-motor center, must be due to activity of the oxygen combined with the iodine in these compounds.

THE ACTION OF HYDROCYANIC ACID ON THE RESPIRATION AND THE ANTAGONISTIC ACTION OF SODIUM IODOSOBENZOATE¹

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Hydrocyanic acid is known to depress physiological oxidations as was first proved by the classical work of Geppert.² Many investigators, beginning with Schönbein,³ have shown that hydrocyanic acid inhibits the activity of the oxidizing enzymes. This is the only fundamental chemical process occurring in living matter which is known to be influenced by this substance and since its physiological and toxicological effects can be readily and satisfactorily explained on the basis that its effects are due to depression of physiological oxidation, it would be a purely gratuitous assumption to suppose that its effects result from changes in any other chemical activity in the cells. We therefore conclude, that in the light of present knowledge, the effects of hydrocyanic action on the function of any cell is the expression of the reaction of that cell to diminished oxidation. Therefore if sodium iodosobenzoate is capable of accelerating physiological oxidation we should expect to find an antagonism between hydrocyanic acid and iodosobenzoate.

The effect of hydrocyanic acid on the respiration is now universally recognized as central. It is unaffected by section of the vagi or by the administration of atropine.⁴ It is well known that hydrocyanic acid is one of the strongest stimulants for the

¹ The expenses of this research have been partly defrayed by a grant from the Rockefeller Institute for Medical Research.

² *Zeitschrift f. klin. Med.*, 15-208, 307 (1889).

³ *Zeitschrift f. Biol.*, 3-140, 325 (1867).

⁴ Boehme and Knie: *Arch. f. exper. Pathol. und Pharmacol.*, 2, 129 (1874).

respiratory center.⁵ The injection of threshold doses intravenously increases the rate and depth of the respirations. When larger quantities are injected there is often noted a slowing of the respiratory rate and a great increase in the depth of the respirations. Still larger quantities cause depression and paralysis of the respiration and death soon ensues. These results have been obtained by a great many investigators and are among the most constant responses of the vital mechanism to drugs. We have, however, noted an interesting phenomenon in the response of the respiratory center to hydrocyanic acid to which attention has not previously been called.

When somewhat more than the threshold dose of hydrocyanic acid is injected intravenously into rabbits we have noted that there is complete stoppage of the respiration for periods varying in our experiments from a few seconds up to 230 seconds and terminating with spontaneous return of the respiration. The rabbits were anaesthetized by giving 0.1 gram chloral and 0.5 gram urethane per kilo subcutaneously and ether was used during the operative procedures. The respiration was recorded by means of a tambour connected with the tracheal tube by means of a T-tube and in most of the experiments Head's method of recording the respiration from the isolated strip of the diaphragm was also employed.

In order to obtain complete temporary stoppage of the respiration, it is necessary to inject from 0.5 to 1 cc. $\frac{N}{25}$ solution per kilo of hydrocyanic acid or sodium cyanid (about 1 mg. hydrocyanic acid or about 2.0 mg. sodium cyanid). The injection should be made rapidly, and it is better to plunge the needle of the hypodermic syringe directly into the vein rather than to connect the syringe to the vein by means of a canula, as the latter delays the injection and requires washing out. In most cases the stoppage occurs in passive expiration but it may occur in any phase of the respiration. If it does occur in active inspiration or expiration the position of passive expiration is gradually assumed during the stoppage. It is difficult or impossible to

⁵ Heinz: *Handbuch der experimentellen Pathologie und Pharmakologie*. Jena, 1906, vol. 2, p. 588.

bring on the respiratory pause without at least a very brief preliminary stage of stimulation. If a small portion reaches the respiratory center first it results in a stage of stimulation, the duration of which depends on the time required for the full dose to reach the center. In order to produce the temporary paralysis it is necessary for the full dose to be injected within a few seconds. The larger the dose, up to the lethal dose, and the more rapidly it is given, the longer will be the respiratory pause. Respiratory paralysis of 1 to 2 minutes duration can be obtained almost without exception. The temporary paralysis of the respiration is independent of the vagi as it occurs whether they are intact or cut. The type of respiration noted on recovery from the paralysis varies somewhat with different individuals. In some cases the first respirations are very small and gradually increase in size until they are many times larger than the normal respirations. During this period the rate is very slow. The respirations then gradually decrease in size and increase in rate until the normal is reached. The return to normal requires 15 to 20 minutes or more. The circulatory phenomena associated with the period of apnoea are interesting and important in the interpretation of the mode of action of hydrocyanic acid. Within 2 to 5 seconds following the injection the heart rate is very much decreased, due in part to stimulation of the cardio-inhibitory apparatus centrally, and in part to direct action on the heart. This is shown by the fact, that if both vagi are cut during the stage of exceedingly slow heart rate, the heart rate increases markedly but does not return to the normal rate. Hence the cardio-inhibitory center is largely but not entirely responsible for the slow heart rate. If atropine is now given in quantities sufficient to paralyze the vagal endings the heart rate does not increase. Hence the residual slowing must be attributed to the direct action of hydrocyanic acid on the heart.

The slow pulse may continue throughout the period of respiratory paralysis and for three or four minutes after the return of the respiration when it gradually becomes normal.

In spite of the great slowing of the heart rate the blood pressure rose 40 mm. in one case due to stimulation of the vaso-motor

center. In another experiment the blood pressure, after a very small and transitory rise, fell 40 mm. indicating that the vaso-motor center, like the respiratory center, was much depressed. The return to normal was very gradual and required several minutes. The work clearly shows that the respiratory center is much more sensitive to hydrocyanic acid than the cardio-inhibitory or the vaso-motor centers. In threshold doses the respiration is affected before the other centers show any response whatever. Larger doses are usually required to slow the heart than to stimulate the vaso-motor center. When excessive doses are used the respiration, having passed through a short stage of stimulation, is paralysed temporarily while the vaso-motor center and cardio-inhibitory center are still in the stage of stimulation. The relative sensitiveness of these three centers to hydrocyanic acid is of much interest as it corresponds exactly with what has been found when the medulla is rendered anaemic.⁶

In connection with the power of hydrocyanic acid to cause temporary stoppage of the respiration it is of interest to review the action of other substances which have a similar effect.

A number of substances are known which cause temporary stoppage of the respiration. Tappeiner⁷ in 1896 studied the action of phenylmethyl-isoxazol-chlormethylate and also that of certain pyrazol derivatives on the circulation and respiration. The first mentioned substance causes a temporary stoppage of the respiration, lasting in one case as long as 80 seconds. Tappeiner states that the respiration stops in active expiration.

Each injection is followed by a very marked slowing of the pulse rate which ceases after section of the vagi and is therefore due to stimulation of the vagal center. The blood pressure always rose from 10 to 20 mm. following each injection, due to stimulation of the vaso-motor center which more than compensated for the slowing of the pulse. Tappeiner found that the effects of the drug resemble the reflex described by Kratschmer.⁸ Kratschmer showed that the injection of irritating vapor into

⁶ J. A. E. Eyster: Jour. of Exp. Med., 8, 565 (1906).

⁷ Arch. f. exp. Path. u. Pharmacol., 37-325 (1896).

⁸ Sitzungsberichte d. Wiener Akad., 62-147 (1870).

the nostrils of the rabbit causes cessation of the respiration in active expiration, a rise in the arterial blood pressure and a slowing of the pulse. Tappeiner used only rabbits in his studies and found that cocainization of the nasal mucosa prevents the drug from exhibiting its characteristic effects. He concluded that the action of phenylmethyl-isoxazol-chlormethylate is peripheral, causing a specific stimulation of the nerve endings of the ophthalmic branch of the fifth nerve in the nasal mucosa.

Jodlbauer,⁹ in Tappeiner's laboratory, studied the action of tetramethyl-ammonium chloride given intravenously to rabbits and found that it causes a respiratory stoppage of 4 to 15 seconds duration, depending on the dose. He showed that the stoppage occurs in strong expiration and can be prevented by cocainizing the nasal mucosa. There is stimulation of the vagus centrally and peripherally and of the vaso-motor center.¹⁰ The Kratschmer reflex fails entirely in dogs and is incomplete in cats and he finds that the drug cannot cause cessation of the respiration in either dogs or cats. He therefore concludes that tetramethyl-ammonium chloride acts by stimulating the endings of the ophthalmic branch of the trigeminus as had been previously found by Tappeiner for the quaternary base investigated by him. Pohl¹¹ found that certain quaternary papaverin bases injected intravenously cause stoppage of the respiration in expiration. He states that soon after the respiratory stoppage asphyxial phenomena occur, vagal pulse, fall of arterial blood pressure and convulsions, and the animal rapidly dies. If the dose is small the respiratory pause is only short and respirations return at first shallow but gradually become normal. He found that the stoppage of the respiration occurs after cocainizing the nasal mucosa and after sectioning the ophthalmic branches of the trigeminus on both sides. Hence he concludes that the drug acts centrally. He found that tetramethyl-ammonium chloride also causes stoppage of the respiration after section of the ophthalmic branch of the

⁹ Arch. internation. de Pharmacodyn. et de Thérapie, 7-187 (1900).

¹⁰ It is clear the stimulation of the vagal endings could not be due to the Kratschmer reflex.

¹¹ Arch. internat. de Pharmacodyn. et de Thérapie, 13-479 (1904).

trigeminus on both sides and concludes that it acts centrally and not peripherally as found by Jodlbauer. Pohl's work leaves little room for doubt that these substances act centrally.¹² Marshall¹³ found that protocatechyl-tropeine in doses of 0.005 to 0.01 gram causes paralysis of the respiration when injected intravenously into anaesthetized rabbits or cats. Complete stoppage usually occurred in 10 seconds after beginning the injection and lasted 4 to 20 seconds according to the dose. Frequently after 0.01 gram the respirations did not return. The respiration ceases in the expiratory phase. Marshall further found that protocatechyl-tropeine still causes the characteristic stoppage of the respiration after excision of the brain above the pons and excision of both fifth nerves. The drug is active in cats in which animals the Kratschmer reflex is not complete so far as the circulation is concerned, although they show stoppage of the respiration when irritating gases are blown into the nostrils. He concluded that this tertiary base acts centrally. In doses which cause respiratory stoppage, Marshall notes that all of these substances cause a decrease in the pulse rate, but he states that this is independent of the respiratory stoppage because they do not occur synchronously and the relative effects vary with different substances. A rise or a fall of blood pressure may be noted. On comparing the action of these drugs with that of hydrocyanic acid we see that they present the following points in common:

1. All cause temporary stoppage of the respiration in appropriate doses.
2. All are very toxic, death being due to respiratory failure.
3. All cause marked slowing and greatly increased amplitude of the pulse due partially to stimulation of the cardio-inhibitory center.
4. Doses required to cause temporary cessation of the respiration may cause a rise or a fall of blood pressure depending on the extent to which the stimulation of the vaso-motor center is capable of compensating for the decreased output of the heart.

¹² Quite recently Marshall has repeated the work of Pohl on tetramethyl ammonium chloride with the same results.

¹³ Jour. of Physiol., 38; Proc. of the Physiol. Soc., lxxxiv, June 5, 1909.

There is, therefore, a very close correspondence between the respiratory and circulatory effects of phenyl-methyl-isoxazol-chlormethylate, tetramethyl-ammonium chloride, the quaternary bases studied by Pohl, protocatechyl-tropeine and hydrocyanic acid. Hydrocyanic acid, in smaller doses than are required to produce temporary respiratory paralysis, is a powerful stimulant for the respiratory center. Jodlbauer notes that tetramethyl-ammonium chloride injected subcutaneously in rabbits increases the rate and depth of the respirations. He found that this substance acts on dogs and cats only as a respiratory stimulant. Very recently Marshall¹⁴ has investigated the action of tetramethyl-ammonium chloride on the respiration. He found that it causes a transient stimulation of the respiratory center increasing both the rate and depth of the respirations in many decerebrated animals. He concludes that the temporary paralysis of the respiration under this substance is due mainly to a paralyzing action on the motor-endings of the respiratory muscles. Unfortunately it is not recorded whether the other drugs which temporarily paralyze the respiration, stimulate it when smaller doses are used. Tappeiner states that phenylmethylisoxazol-chlormethylate often causes convulsions during the respiratory paralysis. This is also occasionally seen when hydrocyanic acid is injected in the doses which we have recommended for producing temporary respiration paralysis. It would seem, therefore, to be highly probable that the other drugs mentioned act in the same manner as hydrocyanic acid, namely, by retarding physiological oxidation. We have found, however, that tetramethyl-ammonium chloride does not retard the direct guaiacum reaction of the extract of potato peel nor does it interfere with the guaiacum peroxidase reaction of human blood or saliva. It does not inhibit the catalytic decomposition of hydrogen peroxide by human blood. In all of these respects it differs markedly from hydrocyanic acid. If this substance does act by inhibiting oxidation in the cells of the medullary centers, either it must be specific for the oxidases of these cells, or it must act through an entirely different mechanism than the oxidizing enzymes.

¹⁴ Journal of Physiol., 42; Proc. Physiol. Soc., p. xxxvii, June 3, 1911.

THE ANTAGONISM BETWEEN HYDROCYANIC ACID AND IODOSOBENZOIC ACID

It is difficult to study antagonism to a substance like hydrocyanic acid which acts with such great rapidity, because the antagonist has to be administered either previously to or simultaneously with the hydrocyanic acid. Our method of studying the antagonism was as follows. Cats anaesthetized with ether were used in the study, as they proved to be better adapted to this work than dogs or rabbits. The blood pressure was recorded from the carotid artery. The respiration was recorded by a tambour connected with the tracheal tube and also directly from the diaphragm by taking a stitch in the diaphragm with a silk thread and attaching this to a receiving tambour. The movements of this tambour were registered by a recording tambour, air transmission being used. Canulas were inserted into the femoral veins on both sides. One canula was used for the injection of hydrocyanic acid and the other for the injection of sodium iodosobenzoate. Starting with very small doses and increasing gradually, the minimal amount of each substance required to produce its characteristic respiratory effects was found. For hydrocyanic acid this consisted of an increase in the rate and depth of the respiration, while for iodosobenzoate the production of an apnoeic period of 10 to 15 seconds duration was taken as the criterion. After allowing a sufficient time to elapse both substances were injected simultaneously and their effects noted. The experiments showed that there exists a very distinct antagonism between the two substances in their effects on the respiration.

The only objection to this method of determining the antagonism between the substances is that it does not show definitely whether the antagonism is pharmacological or chemical, that is to say, whether they act oppositely upon the respiratory center or mutually destroy one another in the blood stream. It is possible that the substances react in the blood stream, the hydrocyanic acid being oxidized and the iodosobenzoate reduced. We believe, however, that this reaction could hardly be expected to occur so quickly and we regard it as more probable that the antagonism

is pharmacological. This result then rather corroborates the view that sodium iodosobenzoate increases physiological oxidation. The protocells of two of the experiments showing the antagonism follow. In Experiment II we were fortunate enough to hit upon doses which almost completely neutralized each other.

Experiment I. Male cat, weight 4.3 kilos. Ether anaesthesia

TIME	DRUGS	RESPIRATIONS		BLOOD PRESSURE	PULSE RATE	REMARKS
		Rate	Amplitude			
<i>hrs, m.</i>			<i>mm.</i>			
4 12½		48	2.5	147	186	
13	1 cc. $\frac{N}{135}$ HCN	56	6.5	154	186	Injection into right femoral.
15½		44	2.5	112	188	
16	1 cc. $\frac{N}{135}$ HCN	44	6.0	120	184	
17½		44	3.5	107	194	
18	2 cc. $\frac{N}{135}$ HCN	64	12.5	115	192	2 cc. of $\frac{N}{135}$ HCN proved to be the threshold dose for this animal.
22		32	7.5	100	180	Changed respiration adjustment.
22½	1 cc. $\frac{N}{100}$ iodoso	24	9.5	104	180	Injection into left femoral.
24		26	9.5	114	182	
24½	2 cc. $\frac{N}{100}$ iodoso			120	186	9½ seconds after injection there was complete cessation of the respiration lasting 14½ seconds.
25		29	11.0	120	180	
40½		24	8.5	134	184	
41	2 cc. $\frac{N}{135}$ HCN 2 cc. $\frac{N}{100}$ iodoso	25	8.5	134	185	12 seconds after injection complete cessation of respiration for 12 seconds.
45½		26	8.5	104	188	
46	2 cc. $\frac{N}{54}$ HCN 2 cc. $\frac{N}{100}$ iodoso	26	11.0	101	198	15 seconds after injection complete cessation of respiration for 5 seconds. Following this the respiration became very fast and irregular in amplitude.

Experiment II. Cat, female, weight 3.52 kilos. Ether anaesthesia

TIME	DRUGS	RESPIRATION		BLOOD PRESSURE	PULSE RATE	REMARKS
		Rate	Amplitude			
<i>hrs. m.</i>			<i>mm.</i>			
10 6½		40	5.5	134	220	
7	2 cc. $\frac{N}{100}$ iodoso	39	5.5	143	228	Injected into left femoral vein.
24½		28	3.0	149	208	
25	2 cc. $\frac{N}{50}$ iodoso	20	3.0	148	208	Apnoea of 6 seconds.
26½		32	3.0	146	220	
27	1 cc. $\frac{N}{20}$ iodoso	12	3.0	152	216	Apnoea of 8 seconds duration.
29		26	3.5	146	220	
29½	1 cc. $\frac{N}{20}$ iodoso			154	212	Apnoea of 18 seconds. See fig. 1.
32		32	4.5	138	224	
32½	2 cc. $\frac{N}{135}$ HCN	36	5.5	139	216	Injected into right femoral vein.
39		32	3.5	148	204	
39½	1 cc. $\frac{N}{54}$ HCN	36	4.5	147	208	
40		29	3.5	147	206	
40½	1 cc. $\frac{N}{54}$ HCN	34	7.0	139	208	
41		30	4.0	136	212	
41½	2 cc. $\frac{N}{54}$ HCN	38	10.0	128	210	Marked dyspnoea. See fig. 2.
47½		24	3.0	134	184	
48	1 cc. $\frac{N}{20}$ iodoso 4 cc. $\frac{N}{54}$ HCN	18	3.0	124	176	There were a few very shallow respirations but neither apnoea nor dyspnoea developed. Within a half minute the respirations were normal in rate and depth. See fig. 3.

SUMMARY

1. Hydrocyanic acid administered intravenously to rabbits in doses of 0.5 to 1 mg. per kilo or sodium cyanide in doses of 1 to 2 mg. per kilo causes temporary stoppage of the respiration

in passive expiration for periods varying from a few seconds to nearly four minutes. The injection must be made rapidly.

2. The respiratory center is more sensitive to hydrocyanic acid than the vaso-motor center and the latter is apparently more sensitive than the cardio-inhibitory center.

3. The action of hydrocyanic acid on the respiration and circulation resembles so closely that of certain other drugs which cause temporary stoppage of the respiration (phenylmethylisoxazol-chlormethylate; Tappeiner), tetramethyl-ammonium chloride (Jodlbauer), quaternary paverin bases (Pohl) and protocatechyl-tropeine (Marshall) that it suggests the possibility that these drugs owe their activity as does hydrocyanic acid to their power to inhibit physiological oxidation in the cells upon which they act.

4. We have established that a definite antagonism exists between hydrocyanic acid and sodium iodosobenzoate in regard to the respiratory center. This antagonism would further indicate that iodosobenzoate is capable of accelerating normal physiological oxidation.

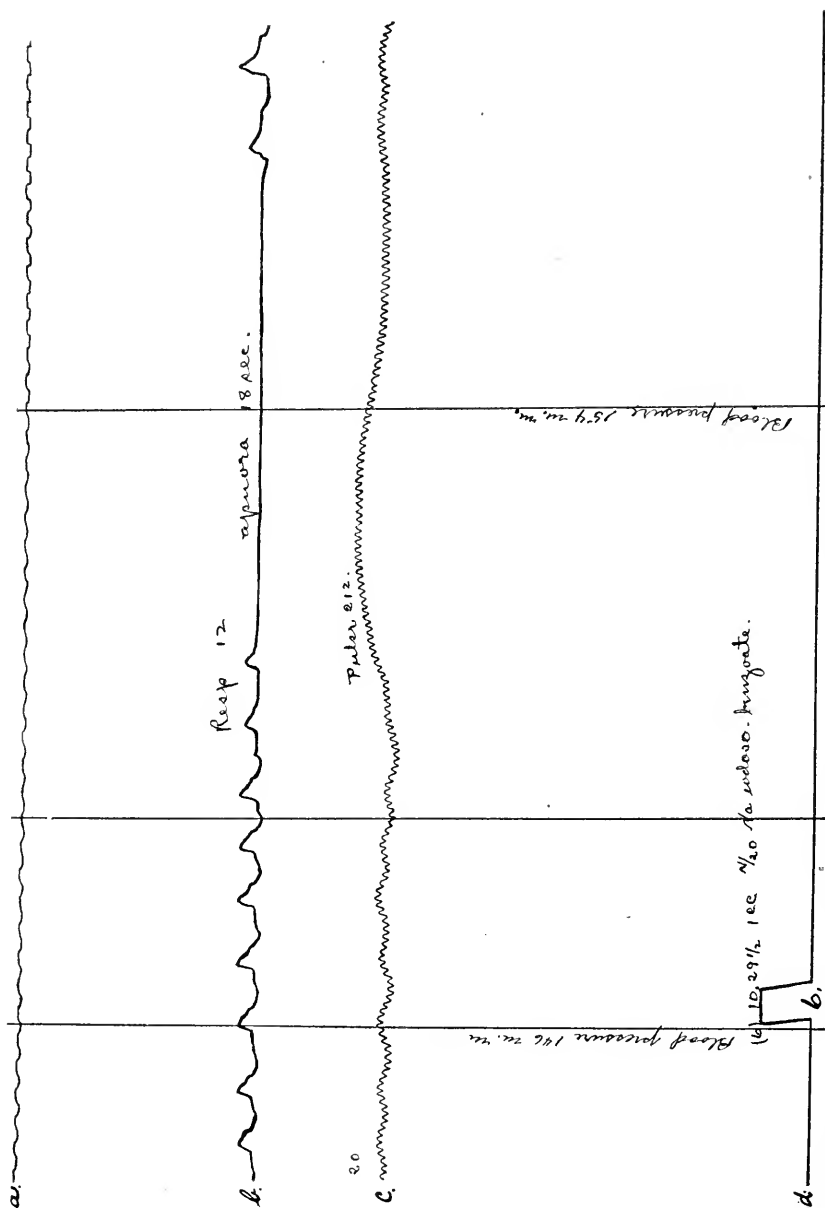


Fig. 1. Female cat, 3.52 K. (See experiment 2.) a. Time record in seconds. b. Respiratory record from tracheal tube, upstroke expiration. c. Blood pressure from Carotid. d. Signal pen and zero pressure. At 6, 1 cc. $\frac{N}{2}U$ sodium iodosobenzoate injected into left femoral vein. Read from left to right.

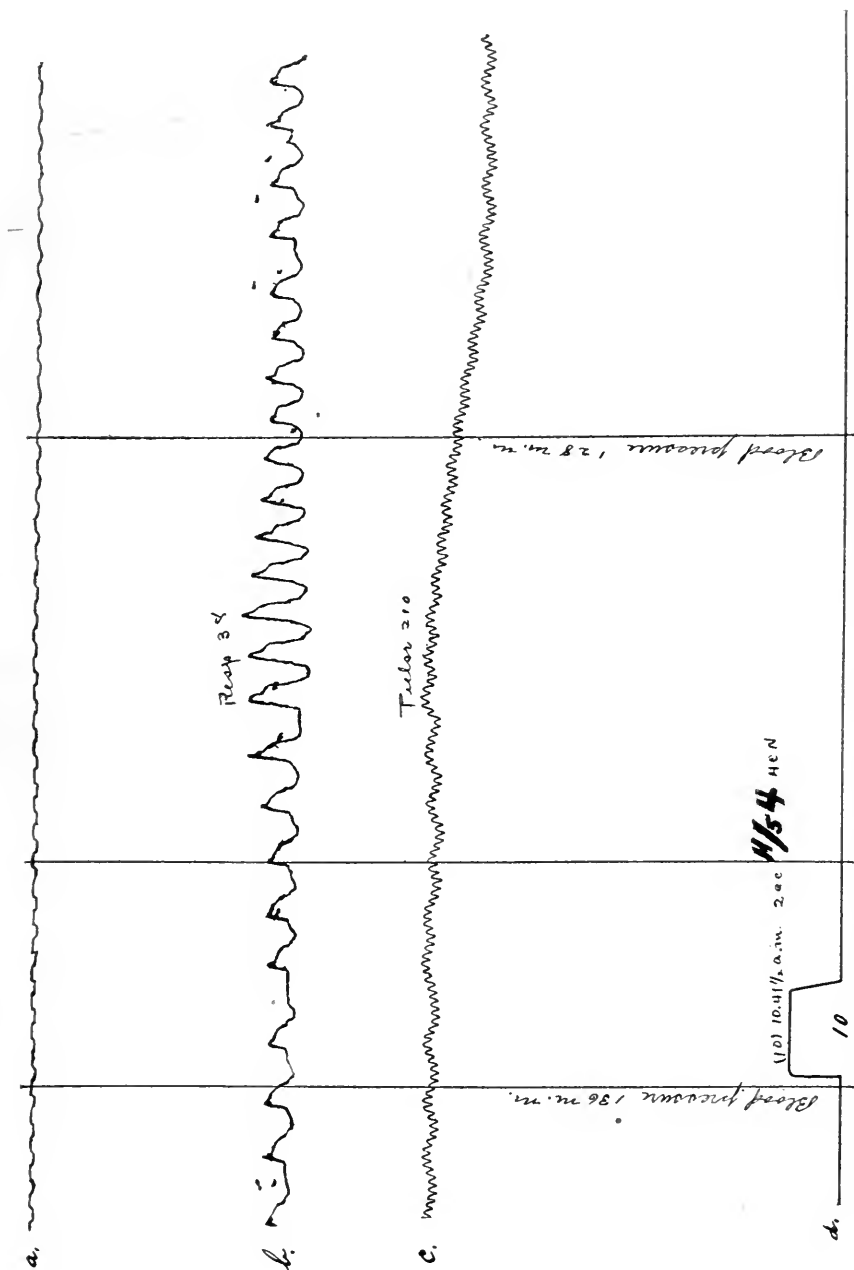


Fig. 2. From same experiment as Fig. 1. At 10, 2 cc. $\frac{N}{4}$ hydrocyanic injected into right femoral vein.

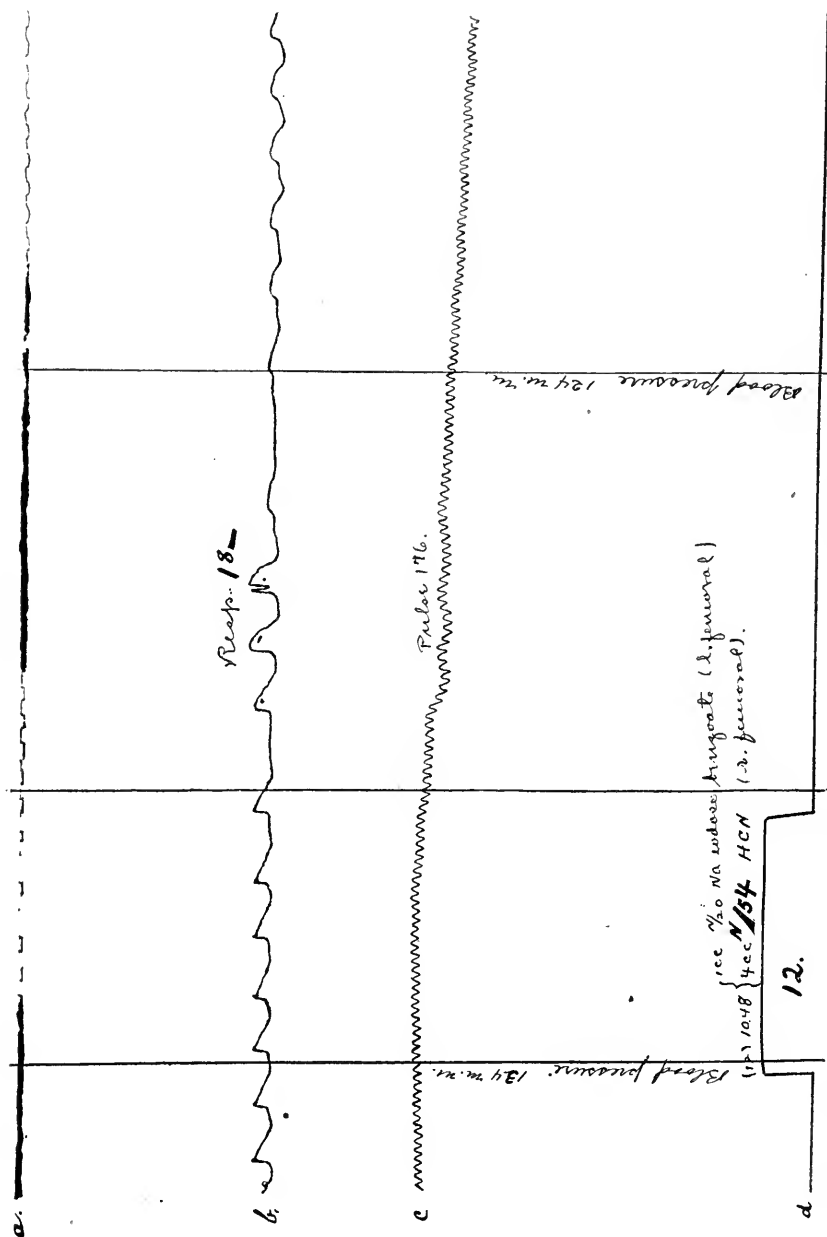


Fig. 3. From same experiment as Figs. 1 and 2. At 12, 1 cc. $\frac{N}{54}$ sodium iodosobenzoate injected into left femoral and 4 cc. $\frac{N}{54}$ hydrocyanic acid injected into right femoral vein.

ON THE ANTISEPTIC AND BACTERICIDAL ACTION OF THE SODIUM SALTS OF IODBENZOIC, IODOSOBENZOIC AND IODOXYBENZOIC ACIDS¹

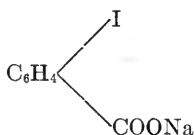
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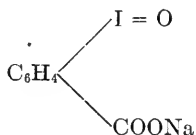
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It was stated by Loevenhart and Grove², that the substances mentioned in the title of this paper were studied in the hope that they would prove useful therapeutically as intravenous antiseptics. Before studying their value as intravenous antiseptics it seemed desirable to ascertain their value as antiseptics in vitro and it is only with this phase of the subject that this communication deals.

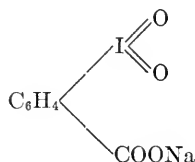
The formulae of these substances are as follows.³



Sodium iodbenzoate



Sodium iodosobenzoate



Sodium iodoxybenzoate

It will be noted that the iodbenzoate contains no active oxygen and is not an oxidizing agent. Iodosobenzoate has an atom of very active oxygen connected with the iodine and contains 6.06 per cent of active oxygen. Iodoxybenzoate, with two atoms of active oxygen bound to the iodine, contains 11.43 per cent of active oxygen. A comparison of the antiseptic and bactericidal

¹ The expenses of this research have been partially defrayed by a grant from the Rockefeller Institute for Medical Research.

² This Journal (Vol. III, p. 101).

³ An account of these substances and the method of preparing them has already been presented by Loevenhart and Grove.

power of these three substances it seemed would throw interesting light on the bactericidal power of oxygen in active form, because the bactericidal power of iodosobenzoate and iodoxybenzoates could differ widely from that of iodbenzoic acid only in consequence of the oxidizing action of the former substances. The reasons for attributing the biological effects of iodosobenzoic and iodoxybenzoic acids, in so far as they differ from the effects of iodbenzoic acid, to the active oxygen, have been discussed by Loevenhart and Grove.

It is especially desired to emphasize the point that we were interested in the bactericidal action of these compounds as active oxidizing agents and not as members of the group of iodine-containing bactericides. Among the most powerful bactericidal substances known are to be found several whose activity depends on their power as oxidizing agents. Among these may be mentioned ozone, benzoyl acetyl peroxide, chlorine, bromine, iodine and the persulphates. Among the less powerful antiseptics of this series may be mentioned hydrogen peroxide and benzoyl peroxide.

It seemed, therefore, that the iodoso- and iodoxybenzoates would prove to possess considerable strength as disinfectants whereas iodbenzoate would be found to be comparatively inert. The experiments proved that this is true.

Methods: In comparing the antiseptic and bactericidal action of substances, it is necessary in order to obtain accurate results to maintain the following conditions: (1) A constant temperature. Throughout this work the organisms were exposed to the bactericides at the body temperature; viz., 37°, because we were interested in these substances as intravenous antiseptics. (2) To use the same strain of organisms throughout the series and to employ as nearly as possible the same number of organisms in each experiment. (3) To employ exactly the same nutrient medium throughout the series. All of the conditions were carefully controlled. A slight modification of the Rideal-Walker⁴ method was employed. The organisms studied were *B. typhosus*, *B. coli*, *B. pyocyaneus* and *Staphylococcus aureus*. The

⁴ Rideal and Walker: Jour. Roy. San. Inst., 24, 424. (1903).

cultures employed were in all cases prepared by inoculating broth tubes from slant agar growths and incubating twenty-four hours at 37°. The tubes were then kept at room temperature until used, which in most cases was not over twelve to sixteen hours.

The bacteria were exposed to the action of the salts in ordinary broth and in the case of *B. typhosus* and *B. coli* also in glucose litmus broth (ordinary broth containing 0.1 per cent azolitmin and 1 per cent glucose). In the former case growth was indicated by the tubes becoming turbid and in the latter case by the development of acid which changed the litmus from blue to red. The methods were equally sensitive and equally satisfactory, the results being practically the same in all cases in which they were compared.

In test tubes were placed 8 cc. of broth and 2 cc. of the solution of the salt to be studied. The solution of the salt used was five times as concentrated as it was desired to study. Thus when it was desired to study a salt in $\frac{N}{1000}$ solution, 2 cc. of a $\frac{N}{200}$ were added to 8 cc. of broth. The tubes were inoculated with carefully measured quantities of the broth cultures previously described. In nearly all cases 0.04 cc. of the twenty-four-hour broth culture was placed in the test tube with the disinfectant mixture. The inoculated tubes were then placed in the incubator at 37° for seventy-two hours. This procedure merely determined the antiseptic action of the substances. In order to determine whether the substances had killed the bacteria or had simply prevented their growth, subcultures were made after twenty-four hours incubation from these tubes into fresh litmus or plain broth, using from two to five loopfuls of material. It was shown by separate experiments that the amount of the substances carried over into the second tube was entirely without effect on the growth of bacteria. The tubes from which the subcultures were made were always shaken before inoculating from them. The subcultures were always observed for three days before pronouncing them sterile. We have simply determined, therefore, the concentration of the salts required to prevent growth for three days and also the concentration required to sterilize the tubes in twenty-four hours at 37°.

Series 1. Sodium iodosobenzoate, *B. typhosus*, Glucose litmus broth. 0.04 cc. of a twenty-four-hour broth culture was used in inoculating the first set of tubes. Temperature 37°

	IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	SUBCULTURES MADE AFTER TWENTY-FOUR HOURS—TWO LOOPFULS	AFTER SEVENTY-TWO HOURS
A.....	None	Red	A ₁	Red
B.....	$\frac{N}{100}$	Blue	B ₁	Blue
C.....	$\frac{N}{300}$	Blue	C ₁	Blue
D.....	$\frac{N}{1000}$	Blue	D ₁	Blue
E.....	$\frac{N}{3000}$	Red	E ₁	Red

This series shows that sodium iodosobenzoate in $\frac{N}{1000}$ solution kills *B. typhosus* in twenty-four hours at 37°.

Series 2. Sodium iodosobenzoate, *B. typhosus*, plain broth. 0.04 cc. of a twenty-four-hour broth culture used

	IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	SUBCULTURES MADE AFTER TWENTY-FOUR HOURS—TWO LOOPFULS	AFTER SEVENTY-TWO HOURS
A.....	None	Turbid	A ₁	Turbid
B.....	$\frac{N}{1000}$	Clear	B ₁	Clear
C.....	$\frac{N}{2000}$	Clear	C ₁	Clear
D.....	$\frac{N}{4000}$	Turbid	D ₁	Turbid
E.....	$\frac{N}{8000}$	Turbid	E ₁	Turbid

A large number of similar experiments were performed, nearly all agreeing with Series 1 that $\frac{N}{1000}$ iodosobenzoate kills *B. typhosus* under these conditions.

Series 3. All conditions the same as in Series 1 except that *B. coli* was used

	IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	SUBCULTURES MADE AFTER TWENTY-FOUR HOURS—TWO LOOPFULS	AFTER SEVENTY-TWO HOURS
A.....	None	Red	A ₁	Red
B.....	$\frac{N}{100}$	Blue	B ₁	Blue
C.....	$\frac{N}{300}$	Blue	C ₁	Blue
D.....	$\frac{N}{1000}$	Blue	D ₁	Red
E.....	$\frac{N}{3000}$	Red	E ₁	Red

In this series *B. coli* was killed by sodium iodosobenzoate in a concentration between $\frac{N}{300}$ and $\frac{N}{1000}$ in twenty-four hours at 37°. Numerous other experiments showed, however, that *B. coli* is killed by $\frac{N}{1000}$ iodosobenzoate under conditions as shown in Series 4.

Series 4. Sodium iodosobenzoate, B. coli, plain broth. Condition same as in Series 3

	IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	SUBCULTURES MADE AFTER TWENTY-FOUR HOURS—THREE LOOPFULS	AFTER SEVENTY-TWO HOURS
A.....	None	Turbid	A ₁	Turbid
B.....	$\frac{N}{1000}$	Clear	B ₁	Clear
C.....	$\frac{N}{2000}$	Turbid	C ₁	Turbid
D.....	$\frac{N}{4000}$	Turbid	D ₁	Turbid
E.....	$\frac{N}{8000}$	Turbid	E ₁	Turbid

Series 5. Sodium iodosobenzoate, Staphylococcus aureus, plain broth. 0.042 cc. of a twenty-four-hour broth culture used; 37°

	IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	SUBCULTURES MADE AFTER TWENTY-FOUR HOURS—TWO LOOPFULS	AFTER SEVENTY-TWO HOURS
A.....	None	Turbid	A ₁	Turbid
B.....	$\frac{N}{100}$	Clear	B ₁	Clear
C.....	$\frac{N}{300}$	Clear	C ₁	Clear
D.....	$\frac{N}{1000}$	Clear	D ₁	Clear
E.....	$\frac{N}{3000}$	Turbid	E ₁	Turbid

This series shows that sodium iodosobenzoate in $\frac{N}{1000}$ solution kills *Staphylococcus aureus* in twenty-four hours at 37°.

Series 6. Sodium iodosobenzoate, B. pyocyaneus, plain broth. 0.04 cc. of twenty-four-hour broth culture used; 37°

	IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	SUBCULTURES MADE AFTER TWENTY-FOUR HOURS—TWO LOOPFULS	AFTER SEVENTY-TWO HOURS
A.....	None	Turbid	A ₁	Turbid
B.....	$\frac{N}{100}$	Clear	B ₁	Clear
C.....	$\frac{N}{300}$	Clear	C ₁	Clear
D.....	$\frac{N}{1000}$	Slightly turbid	D ₁	Turbid
E.....	$\frac{N}{3000}$	Turbid	E ₁	Turbid

Hence sodium iodosobenzoate kills *B. pyocyaneus* in a concentration between $\frac{N}{300}$ and $\frac{N}{1000}$ in twenty-four hours at 37°.

Series 7. A comparison of the germicidal action of the sodium salts of iod-, iodoso-, and iodoxybenzoates on B. typhosus. The organisms were exposed to the antiseptic in plain broth for twenty-four hours at 37° in the usual way at the end of which time subinoculations were made into glucose litmus broth. 0.04 cc. of the broth culture was used in each of the original tubes. The results of the inoculation of the subcultures alone are given below

IODBENZOATE	AFTER SEVENTY-TWO HOURS	IDOSOBENZOATE	AFTER SEVENTY-TWO HOURS	IDODOXYBENZOATE	AFTER SEVENTY-TWO HOURS
None	Red	None	Red	None	Red
$\frac{N}{100}$	Red	$\frac{N}{500}$	Blue	$\frac{N}{500}$	Blue
$\frac{N}{200}$	Red	$\frac{N}{1000}$	Blue	$\frac{N}{1000}$	Blue
$\frac{N}{500}$	Red	$\frac{N}{2000}$	Red	$\frac{N}{2000}$	Blue
$\frac{N}{1000}$	Red	$\frac{N}{3000}$	Red	$\frac{N}{4000}$	Red

This series shows that $\frac{N}{100}$ sodium iodbenzoate does not kill *B. typhosus* in twenty-four hours at 37° whereas the iodosobenzoate kills in $\frac{N}{1000}$ and iodoxybenzoate in $\frac{N}{2000}$ solution. This series of experiments was repeated several times with the same results. Another series of experiments proved that $\frac{N}{10}$ iodbenzoate is required to kill *B. typhosus* under these conditions.

Series 8. Comparison of iodoso-, and iodoxybenzoates on Staphylococcus aureus. Original tubes and subcultures were made in plain broth. The original tubes received 0.04 cc. of the twenty-four-hour broth culture. Temperature 37°. Observations on the subcultures alone given

IDOSOBENZOATE	AFTER SEVENTY-TWO HOURS	IDODOXYBENZOATE	AFTER SEVENTY-TWO HOURS
None	Turbid	None	Turbid
$\frac{N}{500}$	Clear	$\frac{N}{500}$	Turbid
$\frac{N}{1000}$	Clear	$\frac{N}{1000}$	Turbid
$\frac{N}{2000}$	Turbid	$\frac{N}{2000}$	Turbid
$\frac{N}{3000}$	Turbid	$\frac{N}{3000}$	Turbid

Thus $\frac{N}{1000}$ iodosobenzoate kills *Staphylococcus aureus* in twenty-four hours at 37° whereas $\frac{N}{500}$ iodoxybenzoate failed to kill them under the same conditions. Further experiments proved that under these conditions $\frac{N}{200}$ iodoxybenzoate was required to kill *Staphylococcus aureus*.

Series 9. Comparison of iodoso- and iodoxybenzoates on *B. pyocyaneus*. Conditions the same as in Series 8. Observations on subcultures alone given.

IODOSOBENZOATE	AFTER SEVENTY-TWO HOURS	IODOXYBENZOATE	AFTER SEVENTY-TWO HOURS
None	Turbid (green)	None	Turbid (green)
$\frac{N}{200}$	Clear	$\frac{N}{200}$	Clear
$\frac{N}{400}$	Clear	$\frac{N}{400}$	Clear
$\frac{N}{800}$	Clear	$\frac{N}{800}$	Clear
$\frac{N}{1200}$	Turbid	$\frac{N}{1200}$	Clear
$\frac{N}{1600}$	Turbid	$\frac{N}{1600}$	Turbid

This series shows that iodosobenzoate kills *B. pyocyaneus* in twenty-four hours at 37° in a concentration between $\frac{N}{800}$ and $\frac{N}{1200}$ whereas between $\frac{N}{1200}$ and $\frac{N}{1600}$ iodoxybenzoate kills under these conditions.

These experiments prove that sodium iodbenzoate has very little bactericidal power compared with that of iodoso-, and iodoxybenzoates. Using *B. typhosus* it was found that the three substances stand in the following relation to one another:

	CONCENTRATION REQUIRED TO KILL	RELATIVE BACTERICIDAL ACTION
Sodium iodbenzoate.....	$\frac{N}{100}$	1
Sodium iodosobenzoate.....	$\frac{N}{10000}$	100
Sodium iodoxybenzoate.....	$\frac{N}{20000}$	200

Thus toward *B. typhosus* the substance containing one atom of active oxygen is one hundred times as bactericidal as the mother substance containing no active oxygen. The substance containing two atoms of active oxygen is twice as bactericidal as that containing one atom of active oxygen. The results obtained when *B. coli* was used were the same; i.e., the iodosobenzoate kills in $\frac{N}{10000}$ and iodoxybenzoate in $\frac{N}{20000}$ solutions, but the concentration of iodbenzoic acid required to kill *B. coli* was not determined. With *B. pyocyaneus* iodosobenzoate is bactericidal in $\frac{N}{800}$ and iodoxybenzoate in $\frac{N}{1200}$ solution. With *Staphylococcus aureus* iodosobenzoate kills in $\frac{N}{10000}$ solution whereas iodoxybenzoate

requires $\frac{N}{200}$ solution to kill under the same conditions. These differences in the relative bactericidal value of iodoso- and iodoxybenzoates towards different organisms are very interesting. It does not in any sense argue against the proposition that the antiseptic action of both is due to their oxidizing action. A great many instances of specificity in oxidations with the simplest chemicals may be pointed out. If A and B each destroy C and D by oxidative processes the fact that C is more sensitive to A than to B does not indicate that D will also be more sensitive to A than to B, indeed the reverse is just as likely to be found true. This statement holds whether C and D are living micro-organisms or relatively simple chemicals.⁵

THE EFFECT OF PROTEINS AND OTHER ORGANIC MATTER ON THE BACTERICIDAL ACTION OF IODOBENZOATE AND IODOXYBENZOATE

It is well known that the presence of proteins greatly reduces the efficacy of most bactericidal substances. The reason for this is that most bactericidal substances react with proteins and it is in consequence of this property that they are bactericidal. Hence when the bacteria are suspended in a medium rich in proteins, the bactericidal substance combines more readily with the proteins outside of the bacteria and their action on the bacteria is thereby greatly reduced. Among the bactericidal substances of this group may be mentioned all of the salts of the heavy metals, formaldehyde, and phenol and its derivatives.

⁵ An interesting instance of this sort of specificity in oxidative processes was pointed out by Joovenhart and Kastle (Amer. Chem. Jour., 29, 432, 1903). They showed that whereas hydrogen peroxide readily oxidizes formic aldehyde, it is incapable of oxidizing formic acid, yet hydrogen peroxide in the presence of platinum black oxidizes formic acid far more readily than it does formic aldehyde. Similarly formic acid is oxidized by mercuric chloride, whereas formic aldehyde is not and yet formic aldehyde is much more readily oxidized by potassium permanganate in the presence of sulphuric acid than is formic acid. These factors seem not to have been sufficiently considered by Cushny, in his work "On the action of oxidizing salts" Arch. f. exper. Path. u. Pharm. Schmiedeberg-Festschrift, 1908, p. 126.

The work of Ehrlich and Bechhold⁶ brought out beautifully the effect of proteins in protecting bacteria from the action of a bactericide whose action in all probability depends on its power to combine with proteins. Ehrlich and Bechhold found that tetrachlor-o-biphenol prevents the growth of *B. diphtheriae* in a dilution of 1:320,000 in the absence of protein, but in the presence of blood its antiseptic action was so reduced that a concentration of 1:10,000 did not entirely prevent growth. Bechhold⁷ succeeded in proving, by means of his gelatin filters, that his substance was combined with the proteids of the blood.

Since proteins do not readily suffer oxidation, and since our substances owe their bactericidal action to their oxidizing power, we were led to believe that proteins would not greatly influence the bactericidal action of iodoso- and iodoxybenzoate. This was found to be the case.

*Series 10. About 300 cc. of blood were drawn aseptically from a large dog. This was kept for forty-eight hours at room temperature, when the serum was pipetted off and placed in the ice chest for twenty-four hours. The serum was heated to 55° for one hour in order to destroy its bactericidal action. The serum was straw colored and contained no haemoglobin. Each tube contained 5 cc. of serum and 1 cc. of iodoxybenzoate of proper strength to give the concentration stated in the table. Each tube was inoculated with 0.0194 cc. of a broth culture of *B. typhosus*. The sub-inoculations recorded in the table were made into glucose litmus broth after the original tubes had been incubated twenty-four hours at 37°*

	IODOXYBENZOATE	SUBCULTURES AFTER SEVENTY-TWO HOURS
A.....	None	Red
B.....	$\frac{N}{600}$	Blue
C.....	$\frac{N}{1200}$	Blue
D.....	$\frac{N}{2400}$	Blue
E.....	$\frac{N}{4800}$	Red
F.....	$\frac{N}{9600}$	Red

⁶ Zeitsch. f. physiol. Chem., 47, 173 (1906).

⁷ Zeitsch. f. physiol. Chem., 52, 177 (1907).

A similar experiment with unheated serum gave the same result.

Series 11. This series of experiments was carried out as described under Series 10 except that a 5 per cent gelatin solution was substituted for the serum

	IODOXYBENZOATE	SUBCULTURES IN GLUCOSE LITMUS BROTH AFTER SEVENTY-TWO HOURS
A.....	None	Red
B.....	$\frac{N}{200}$	Blue
C.....	$\frac{N}{100}$	Blue
D.....	$\frac{N}{1000}$	Blue
E.....	$\frac{N}{2000}$	Blue
F.....	$\frac{N}{1000}$	Blue

These experiments show that the bactericidal action of sodium iodoxybenzoate is not diminished either by the presence of $83\frac{1}{3}$ per cent dog serum or $4\frac{1}{6}$ per cent gelatin.

SUMMARY

1. Sodium iodoso- and iodoxybenzoates have far greater bactericidal power than sodium iodbenzoate. Toward *B. typhosus* sodium iodosobenzoate is one hundred times and sodium iodoxybenzoate is two hundred times as bactericidal as sodium iodbenzoate. This difference must be attributed to the oxidizing action of the former substances.

2. Toward *B. typhosus* and *B. coli* sodium iodoxybenzoate is twice as strong as sodium iodosobenzoate.

3. Toward *B. pyocyaneus* sodium iodoxybenzoate is about 1.5 times as bactericidal as sodium iodosobenzoate.

4. Toward *Staphylococcus aureus* sodium iodosobenzoate is about five times as bactericidal as sodium iodoxybenzoate.

5. The presence of either $83\frac{1}{3}$ per cent blood serum or $4\frac{1}{6}$ per cent gelatin does not diminish the bactericidal action of sodium iodoxybenzoate.

6. The comparison of the bactericidal action of these three substances furnishes an interesting example of the influences of chemical constitution on bactericidal action.

In conclusion I desire to thank Dr. A. S. Loevenhart for suggesting this work to me and for his assistance in carrying it out.

A NEW WATER SOLUBLE ACTIVE CONSTITUENT OF SQUILLS

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Notwithstanding the extensive therapeutic employment of squill and the pharmacological interest attaching to it on account of its relation to the digitalis series of heart poisons the work published on this subject from the chemical point of view is very scanty.

In 1879 Jarmersted (*Archiv. f. exp. Path. u. Pharm.*, xi, 22) described an amorphous glucosidal toxic product "scillaïn" which was very little soluble in water but readily soluble in alcohol. In the same year E. Merck (*Pharm. Zeit.*, 1879, no. 38) described three active constituents of squill, namely, scillitoxin, scillipicrin, and scillin. Of these scillitoxin may be considered to be practically identical with Jarmersted's "scillaïn," and a dose of from 0.12 to 0.13 mg. injected into a frog was sufficient to cause death. Neither of these products however can be considered to represent the active principle of squill in a pure form.¹ Scillipicrin is very feebly active (10 to 20 mg. being necessary to cause death in the frog) while scillin is even less active. Since these publications no communication of any importance has appeared, although Waliszewski in 1894 (*L'Union Pharm.*, xxxiv, 251) claimed to have isolated three crystallisable active principles which he termed scillinine, scillipicrine, and scillimarine. His work however is unsupported by any experimental detail and no further communication from this source has appeared.

¹A commercial specimen of scillitoxin was easily shown to be far from a pure product. In the first place the product is not wholly readily soluble in absolute alcohol and merely precipitating the alcoholic solution with ether yielded a product which was about twice as toxic as the original preparation.

During the last two years experiments have from time to time been carried out in these laboratories with the object of isolating in a crystalline form the active principle (or principles) of this drug and although the primary object of the investigation has so far not been achieved the results obtained appear to be of sufficient interest and importance to be worthy of record.

The most interesting result of the work has been to show that the activity of squill is due in part at least to a water-soluble glucoside which somewhat resembles the strophanthins from *strophanthus hispidus* and *strophanthus kombe* both from the chemical point of view, with regard to its solubilities, glucosidic nature, and colour reactions, and from the pharmacological side on account of its high degree of toxicity and similarity of action. The minimum lethal dose of this substance is 0.03 mg. for an average sized frog (25 grams) death occurring within three hours after injection, the heart being arrested in systole. This minimum lethal dose is thus about equal to that of either of the strophanthins mentioned above, somewhat less than that of digitoxin in its purest form, and almost half that of apocynamarin the recently isolated crystalline active principle of *apocynum* (Moore, Trans. Chem. Soc., 1909, 95, 735; Finnemore, Proc. Chem. Soc., 1909, 25, 77).

The most suitable method of preparation of this highly toxic substance is set out below. By the method employed the whole of the original activity of the squills tincture was obtained in the form of a solid the toxicity of which was about three times as great as that of Merck's scillitoxin.

Ten litres of concentrated *Tinctura Scillae* ($5 \times$ B.P.) were shaken out six times with, each time, half this volume of a mixture of chloroform (9 volumes) and alcohol (1 volume). The extracts were mixed, washed with small quantities of dilute sodium carbonate solution until the washings were alkaline, then with water until neutral, and finally evaporated to dryness under diminished pressure. There was thus obtained 21 grams of a dark brown solid which was found to represent practically the whole of the original activity of the tincture.

In order to determine the activity of the various fractions produced the following procedure was employed. The minimum lethal dose of each fraction was determined, as that amount of substance per 100 gram of body weight which would cause the death of a frog within three hours after injection into the dorsal lymph sacs, the heart being arrested in systole. These very numerous determinations have in all cases been carried out by Dr. P. P. Laidlaw of these laboratories, to whom the author wishes to express his indebtedness for this very necessary help, and for many valuable suggestions during the course of the investigation. It was thus found that the m. l. d. of the crude product obtained as described above was approximately 0.20 mgs. and, as already stated, represented the original activity of the tincture almost quantitatively.

The solid was dissolved in about 100 cc. of hot absolute alcohol in which it was almost completely soluble. On standing a very small quantity of a substance which proved to be inactive, and was not further examined, separated. The alcoholic solution was poured into about 1500 cc. of dry ether and the bulky precipitate which separated allowed to settle. The ethereal liquid was then syphoned off and the precipitate again dissolved in alcohol and the precipitation repeated. The ethereal solution was taken to dryness, the residue taken up in alcohol and again precipitated by dry ether. In this manner there were finally obtained two fractions: (a), soluble in alcohol and precipitated by ether, an amorphous light coloured, non-hygroscopic, water soluble powder; (b), a resinous product soluble in ether and in alcohol.

Of fraction (a) 4.9 grams were obtained of which the m. l. d. was found to be 0.15 mg. per 100 gram (frog) and which therefore represented about one-third of the original activity. It was observed that the aqueous solution of this substance (a) on warming became opaque owing to the separation of oily drops. The substance was therefore dissolved in about four to five parts of cold water and kept in a boiling water bath for about half an hour when the oily product settled to the bottom of the vessel and the clear supernatant liquid was syphoned off. On evaporation the solution yielded a deep yellow coloured solid which was not

hygroscopic, could be readily powdered and was found to be of a very high degree of toxicity. 0.03 mg. injected into a frog of 25 grams weight produced death within less than three hours, the substance thus possessing an m. l. d. of 0.12 mg. per 100 gram (frog). About 70 per cent of the original water soluble product was completely soluble in hot water.

The final product thus obtained was free from nitrogen, readily soluble in cold water, methyl, ethyl, or amyl alcohol, acetic acid and pyridine, but almost insoluble in ether, chloroform, ethyl acetate and the other usual organic solvents. The aqueous solution possesses an extremely bitter taste. With sulphuric acid the solid gives a brown colouration which on careful dilution gives first a purple violet colour and on further dilution passes through a rose red colour to a green solution from which finally a greyish green flocculent precipitate separates (cf. strophanthin reaction). The aqueous solution of the solid gives no precipitate with solutions of the heavy metals, e.g., mercuric chloride, lead acetate, etc., nor with tannic acid but the substance (again like the strophanthins may be salted out by saturation with ammonium sulphate. Alcoholic solutions treated with alcoholic lead acetate give no precipitate even on long standing. All attempts to crystallise this product have so far failed but experiments in this direction are being continued.

On hydrolysis with dilute acids the solution of this water soluble product becomes turbid very rapidly even at the ordinary temperature and a resinous product separates, while the solution acquires reducing properties which are due to the liberation of a sugar which under the usual treatment yields d-phenyl glucosazone.

The resinous product (b) soluble in ether containing a small proportion of alcohol, which represents approximately two-thirds of the original activity of the squill extract, appears to be a considerably purer form of Merck's scillitoxin. That this is so appears probable since scillitoxin by suitable treatment yields a product very similar in all respects to the resinous product (b). Thus if scillitoxin is dissolved in absolute alcohol (scillitoxin is by no means completely soluble in absolute alcohol) and the alcoholic

solution precipitated by pouring into 10 to 12 volumes of dry ether, the product remaining in solution in the ether-alcohol mixture possesses an m. l. d. which is approximately one-half that of the original scillitoxin. This product is thus of the same order of activity as the resinous product (b), has similar solubilities, viz., readily soluble in absolute alcohol, soluble in alcohol-ether mixture containing 7 to 10 per cent of alcohol, almost insoluble in water. Moreover both preparations on treatment with concentrated sulphuric acid and careful dilution with water give a very distinct green coloration (a reaction which differs from that given by the water soluble product in that there is no initial red or violet colour) similar to that given by digitoxine. It appears unlikely that the toxicity of scillitoxin can be due to the presence of any of the water soluble substance since the preparation is only soluble in water to a very slight extent and the characteristic colour reaction is completely absent.

It seems indeed most probable that the activity of squills is due to the presence of at least two active principles, one of which is very readily soluble in water, the other only very slightly.

ISOLATION OF CAFFEINE FROM SQUILLS

No attempt has been made to carry out a complete chemical investigation of squills, but during the course of the work the interesting discovery was made of the existence of caffeine in the squill bulb.

The isolation of the base was accomplished as follows. The dry powdered squills (10 kilos) were extracted with hot 97 per cent alcohol and the extract concentrated to small bulk (1300 cc.). After filtering from precipitated resin the liquid was completely precipitated by basic lead acetate solution. The excess of lead was removed by means of sulphuric acid and the excess of sulphuric acid by baryta. The clear yellow filtrate was concentrated to a thin syrup (400 cc.) and thoroughly shaken out with amyl alcohol. The alcoholic solution was evaporated to dryness and the gummy residue dissolved in absolute alcohol and taken to dryness. After repeating this treatment a few times a crystalline

solid commenced to separate. The alcoholic solution was then allowed to stand for some time, and the solid then filtered off. After recrystallisation from alcohol, from which it separated in fine needles the substance was found to melt at 229° to 230° . It contained nitrogen was appreciably soluble in water, was not glucosidal but gave rather feeble alkaloidal reactions.

On analysis

0.1288 gave 0.0235 CO_2 and 0.0592 H_2O . $\text{C}=49.8$, $\text{H}=5.1$.

0.1152 gave 27.9 cc. N_2 (moist) at 12° and 770 mm. $\text{N}=28.9$

$\text{C}_8\text{H}_{10}\text{O}_2\text{N}_4$ requires $\text{C}=49.5$, $\text{H}=5.1$, $\text{N}=28.8$ per cent.

whence it was at once seen that the substance was in all probability caffeine. This was confirmed. The substance gave the murexide test, could be sublimed, and when mixed with a specimen of caffeine from another source the mixture melted at 231° .

The total amount of this base present in squills is however very small (approximately 0.01 per cent of the dry bulb) and from the pharmacological point of view is negligible in relation to the well known diuretic effect of the drug.

SUMMARY

The toxicity of squills is in all probability due to the presence of at least two active principles.

1. A glucosidal substance very easily soluble in water, and resembling in many respects the water soluble strophanthin. This substance has been isolated in an approximately pure form but so far has not been crystallised. The minimum lethal dose of the product is 0.03 mg. for a frog of about 25 grams weight, death being produced by the stopping of the heart in systole.

2. A resinous product very slightly soluble in water, readily soluble in alcohol and not precipitated by ether from its alcoholic solution. The m. l. d. of this product is 0.06 to 0.07 mg. per frog.

From the alcoholic extract of the squill bulb a small quantity of caffeine was isolated. The base is present only in very small quantity (0.01 per cent of the dry bulb).

THE PHYSIOLOGICAL ACTION OF PHENOLPHTHALEIN OXIME

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CONTENTS

I. Introduction.....	161
II. Properties of Phenolphthalein Oxime.....	162
III. Methods employed.....	165
IV. Physiological Action of the Oxime.....	165
1. Local action.....	165
2. Action on bacteria.....	165
3. Action on the intestine.....	166
4. Channels of absorption.....	166
From the stomach and intestine.....	166
From subcutaneous tissue.....	170
From the peritoneal cavity.....	170
Intravenous injection, with blood pressure measurements.....	170
5. Antipyretic action.....	171
6. Toxicity.....	174
7. Fate and elimination.....	175
V. Discussion of Results.....	176
VI. Summary.....	180

The phthaleins form an extensive and important series of compounds which have received much more attention from the chemists than from the pharmacologists. Interest in the series from the pharmacological side has been given an additional stimulus by the work of Abel and Rowntree,¹ who have recently brought out some valuable facts regarding the physiological action of some of the phenolphthalein derivatives.

As is well known, phenolphthalein exerts a laxative action, but this effect is of short duration. This is due to the fact that

¹Journ. Pharm. and Exp. Therap., 1, p. 231, 1909.

the drug quickly leaves the body through the feces and urine. In the paper referred to, Abel and Rowntree have shown that the introduction of four chlorine atoms into the phenolphthalein molecule markedly influences the absorption, excretion, and laxative action of the phthalein. They found that after subcutaneous injection of the tetrachlor derivative it is excreted by the bile, is reabsorbed by the large intestine (not by the small), excreted again by the bile, and reabsorbed as before. This interesting cycle is maintained for some days, and a mild, though certain, laxative action accompanies it all the while. The compound does not appear in the urine unless given in large quantities. If, on the other hand, it is given by mouth, none of it appears in the bile or urine, but it exerts a laxative effect similar to that of phenolphthalein itself. No evidence of toxic action was found after either method of administration. The tetrachlor compound promises, therefore, to be a very efficient hypodermic laxative. Other derivatives were found to be less active in this respect.

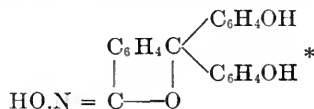
In view of these results obtained by Abel and Rowntree, Professor Orndorff, who has made an extensive study of the chemistry of the phthaleins, suggested that it would be interesting to investigate the action of the *oxime* of phenolphthalein, as it appeared that such a study had not been made. For the investigation he kindly supplied me with this substance, which was prepared in pure form in his laboratory.

PROPERTIES OF THIS OXIME

Physical. This substance has a pale greenish yellow color, somewhat like that of powdered sulphur. When combined with an alkali, such as NaOH, the yellow color becomes intense, and is even more pronounced when the hydrochloride is formed. The oxime melts with decomposition at 212°C . It is soluble in ninety parts of water, and very sparingly soluble in alcohol. In other fluids it is practically insoluble. It crystallizes with great difficulty.

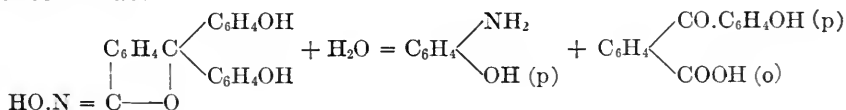
²Friedländer, P.: Ber. d. deutsch. chem. Gesell., 26, p. 174, 1893.

Chemical. The structural formula of this substance is not exactly known, but according to Meyer and Kissen³ it is probably



*In a personal communication to the writer, Professor Orndorff states that from work done in his laboratory he thinks this is probably the correct formula.

It forms weak compounds with acids and alkalis, and when boiled in water in the presence of H_2SO_4 or HCl it readily hydrolyzes, forming p-amino-phenol and p-oxy-o-benzoyl benzoic acid. This hydrolysis will even occur in pure aqueous solution, because at the end of twenty-four hours at room temperature one can demonstrate the presence of free p-amino-phenol. At 38 to 40° C. this decomposition is a little more rapid. The presence of a weak acid, such as 0.2 per HCl , does not accelerate the reaction much, but if one-sixth the volume of conc H_2SO_4 is added it is greatly hastened, p-amino-phenol being detectable within one hour at room temperature. The reaction taking place may be represented thus:



This decomposition is very important in the present research, because it furnishes a method of determining the fate of the oxime in the body. The method depends upon the fact that p-amino-phenol gives the indophenol test, which is exceedingly delicate. The reaction depends upon the oxidation of the p-amino-phenol in the presence of phenol, the end point being the formation of an intensely blue alkaline salt when ammonia is added. Regarding the delicacy of this test for p-amino-phenol, E. Meyer⁴ says that it is possible to obtain the reaction when the dilution of the p-amino-phenol is as great as 1 in 1,600,000. While he does not state that this is true in pure solution in water only, I have found

³Ber. d. deutsch. chem. Gesell., 42, p. 2825, 1909.

⁴Zeit. f. Physiol. chemie., 46, 500, 1905.

that to be the case. In urine, or in extracts of tissues or feces, where body pigments are present, the sensitiveness of the test is greatly reduced. In determining just how delicate the reaction is under various conditions, I have used freshly made solutions of Merck's p-amino-phenol. The test is best made as follows, according to my experience: of the fluid to be examined, one takes about 6 to 8 cc. in a test tube, adds about 1 cc. of conc H_2SO_4 , mixes it thoroughly with the fluid and places the mixture, in boiling water for two to five minutes, or longer (except when dealing with very small quantities of p-amino-phenol, boiling for as long as one hour, even in urine, does no harm.⁵ The mixture is cooled and a few drops of 2 per cent phenol are added and the tube shaken. Next, one adds a drop or two of 1 to 2 per cent potassium bichromate, or chromic acid, and agitates the tube so as to disseminate the oxidizing agent through the upper third of the liquid. After a minute or two, one runs into the top of the mixture some strong NH_4OH by means of a pipette, care being taken not to mix the NH_4OH much with the subjacent fluid. If p-amino-phenol be present, a distinct blue ring will appear at the junction of the acid and alkaline liquids. The blue color will be faint, very distinct or deep indigo according to the amount of p-amino-phenol in the solution. The blue color is the end point to be sought for. The pink or red color which one often sees when the oxidizing agent is added may or may not be due to p-amino-phenol, and when due to this latter substance it may soon be obscured by other oxidation products. In dealing with small quantities of this amino-phenol, it is important not to add too much of the bichromate, as the color of this reagent interferes. The same is true if ferric chloride is used. Furthermore, the ammonia must be added within a minute or two after the oxidizing agent.

By the above procedure I have been able to detect one part of amino-phenol in 4,000,000 parts of water, by using a control tube of water with simply the reagents added, and by viewing the tubes with day light reflected from a white surface. In the urine and other body fluids containing pigments the test is positive in

⁵Unless evaporation and concentration of the urine take place.

dilutions only as high as 1 to 50,000, as a rule, though in some instances I have obtained the reaction in urine containing only one part of p-amino-phenol in 200,000.

METHODS EMPLOYED

The animals used were dogs, cats, rabbits, guinea pigs, white rats, woodchucks, a sheep, and a coyote. One experiment was made upon man. The drug was given by mouth, per rectum, by direct injection into the stomach, and intestines, and by intravenous and hypodermic injections. For administration by the last two methods the oxime was dissolved in 0.2 per cent Na_2CO_3 solution to which 0.9 per cent NaCl had been added. It could only be dissolved in sufficient quantity to be used by means of an alkali, Na_2CO_3 being preferable. After administration, various organs and fluids of the body were examined for the unchanged oxime and for free and combined p-amino-phenol which was found by the method described. The oxime was considered present as such when the tissue extract, made by macerating the finely minced organ for twenty-four to forty-eight hours in weak NaOH solution, gave a good indo-phenol test after boiling with conc H_2SO_4 , a watery extract made in the same length of time giving no such test. If the watery extract did give the test, p-amino-phenol was present, either free or combined. The compound could be split by boiling with acid.

PHYSIOLOGICAL ACTION OF THE OXIME

LOCAL ACTION. The finely powdered oxime was dusted into the eye of a rabbit. It produced no more irritation than a dry, inert powder would. It did not show any irritant action when applied to open wounds. No local action on the mucosa of the alimentary canal could be detected. The lack of an irritant action was probably due to its insolubility.

ACTION ON BACTERIA. Extended experiments upon this line were not made, but it was noticed that body fluids, such as urine, blood, bile, etc., underwent putrefaction rapidly in the presence of the oxime. The conclusion seems justified that it has no anti-putrefactive or antiseptic action.

ACTION ON THE INTESTINE. Early in the work experiments were started to determine whether or not the drug would have a purgative action. The criterion of purgative effect adopted by Abel and Rowntree was used. Dogs were put upon such a régime of diet and exercise that their feces became hard and dry. In producing such a consistency of feces, the addition of bone ash was found helpful. The animals were then fed 0.5 to 1.0 gram of the oxime mixed with the food, and the amount and character of the stools noted, together with time of passage. No constant effect upon the amount, color, consistency, or time of passage of the feces could be observed. Occasionally there seemed to be some softening of the feces, but not enough to call the effect a distinct laxative action. The same experiments were tried upon cats, but the results with these were still less reliable. In man, too, the effect was doubtful. The writer took 0.4 gram of the oxime upon an empty stomach. No action was noticeable. The experiment was not repeated because of the toxicity of the drug, a point which will be discussed later.

Besides administration by mouth the drug was given hypodermically to dogs and cats with no effect upon the bowel.

In this respect, the oxime stands in marked contrast to phenolphthalein itself, and to the tetrachlor compound. As a compound, it is physiologically inert, as shown by the above experiments and by others to be described later.

CHANNELS OF ABSORPTION. 1. *Absorption from the stomach and intestines.*

Experiment I. (a) Three cats were fed the substance mixed with meat, and were killed at the end of $1\frac{1}{2}$, $3\frac{1}{2}$ and 5 hours, respectively. Blood, urine, kidneys, and liver were examined for the oxime and for p-amino-phenol. No trace of either was found.

(b). Four cats were etherized. In two the pylorus was ligatured and 0.5 gram of oxime, suspended in 30 cc. of water, was injected into the stomach of each. They were allowed to recover from the ether and then killed at the end of five and seven hours respectively. No trace of oxime or p-amino-phenol could be found outside of the stomach. In the other two cats, after etherization, the small intestine was ligated at its two ends and a watery

suspension of the oxime was injected into the duodenum. They were allowed to recover from the anesthetic and were killed by ether at the end of five and seven hours. No trace of oxime or p-amino-phenol could be found in urine or tissues.

(c). Four other cats were fed 0.5 gram of oxime at 10 a.m. and 5 p.m. Urine was collected in animal cages. In one cat the urine of the first twenty-four hours gave a faint test for p-amino-phenol, and at end of forty-eight hours the test was certain. It was positive until the end of the fifth day. In two of the cats the test was positive between the second and third days. In a fourth one p-amino-phenol never appeared in the urine. The experiment was repeated on a fifth cat and the test was not positive until the third day. It is evident that in the cat this oxime is absorbed very slowly from the alimentary canal.

Experiment II. Absorption in rabbits. Ten animals were used in this series. In the first six, 0.5 gram to $1\frac{1}{2}$ grams of oxime suspended in water, was given by stomach tube, and urine collected in animal cages, care being taken to avoid contamination with feces. In two rabbits the urine showed the indo-phenol test within $2\frac{1}{2}$ hours; in two others it was found within four and six hours, in first portion passed; in the fifth and sixth no urine was passed until twenty-four hours after giving the drug, when a good indo-phenol test was obtained.

In each animal the character of the urine was changed. Instead of secreting a thick, dark gelatinous fluid, as may often be noticed with rabbits, they passed a light lemon colored urine, of lower specific gravity, and in larger quantities than normal. There was undoubtedly diuresis, which was not due to the water given as the extra urine considerably exceeded in amount the water administered. On account of the yellow color and the intensity of the indo-phenol test, it was concluded that some of the oxime passed into the urine unchanged, but whether it was excreted free, or combined with some body, such as glycuronic acid, could not be determined. The indo-phenol test continued to show in the urine until the fourth, sixth or even seventh day after the oxime was given. In the fifth rabbit it disappeared on the sixth day after the oxime was given, but could still be

detected in the feces. It is evident from these experiments that the oxime is absorbed with comparative rapidity in rabbits.

In order to determine what part of the alimentary canal of the rabbit is most active in absorbing the oxime, experiments were performed in the same manner as with the cats, that is, various parts of the canal were isolated by ligatures. In one the stomach was isolated; in the second, the duodenum; in the third, the lower part of the small intestine; and in the fourth, the large intestine was used. A watery suspension was injected in each case. In the first rabbit no indo-phenol test was obtained in the urine at end of eight hours, when the animal was killed; in the second and third, which were killed at end of $2\frac{1}{2}$ and 5 hours, respectively, the urine showed a distinct reaction; the fourth rabbit, which received a rectal injection, passed urine in twelve hours, and the test was also positive. It appears from these experiments that the oxime is rapidly absorbed from the small intestine of the rabbit, but not from the stomach.

Experiment III. Absorption in man. The writer took 0.4 gram of the oxime by mouth in capsule, at 11:30 a.m. Stomach empty. Urine was collected at end of every hour. At the end of the third hour the sample gave a good indo-phenol test, after boiling with H_2SO_4 . At end of the fourth hour, the color of the specimen was distinctly altered, being of a lemon yellow shade. No albumin was present. No evidence of diuresis was noted, nor any laxative action. The experiment was not repeated, but it was evident that absorption had been fairly rapid, and had probably occurred from the small intestine. Whether any oxime was excreted unchanged could not be determined. The change of color in the urine could not decide the question.

Experiment IV. Absorption in the dog. In the experiments with dogs mentioned earlier in the paper the urine was not collected with any regularity. It was therefore necessary to make further observations upon absorption in dogs. Six in all were used, of which one was a pup a month or two old, the rest being adults.

1. A large dog was fed 0.5 gram oxime, mixed with meat, at 5:45 p.m. It was killed twenty-five hours afterward and urine taken from the bladder. Test for p-amino-phenol was negative.

2. Another large dog was fed 1.5 grams oxime in milk. It was killed twenty-four hours later. Oxime was found all along the small and large intestine, but the urine showed no evidence of absorption.

3. In a third dog it was shown that no absorption occurred from the stomach within six hours.

4. A pup, two months old was given 0.4 gram oxime in water by mouth. The urine gave a good p-amino-phenol test within $2\frac{1}{2}$ hours.

5. Full grown dog was given 0.5 gram of oxime in meat at 11 p.m. Passed no excreta until $33\frac{1}{2}$ hours later. The large quantity urine voided contained trace of p-amino-phenol. Trace in feces also.

6. Full grown dog. Procedure same as in 5. No trace of p-amino-phenol in the urine within forty-eight hours. Trace of oxime in feces.

With the exception of no. 4 absorption has been slow.

Experiment V. Absorption in the coyote (*Canis Latrans*, Say). From the experiments with cats, dogs and rabbits, it was thought that possibly carnivora and herbivora might show a distinct difference in absorption. A coyote was available and was given some oxime mixed with meat, at 8:30 a.m. The first sample of urine was passed at 5:30 p.m., nine hours after receiving the drug. The urine gave a good indo-phenol test. Doubtless absorption had occurred several hours before the sample was voided, and resembled that in the rabbit more than in the cat and dog. Urine was free from p-amino-phenol within 48 hours.

Experiment VI. Absorption in the sheep. One young lamb was used. The result was entirely negative for some reason, no reaction for p-amino-phenol being obtained. The experiment was not repeated.

Experiment VII. Absorption in the woodchuck (*Marmotta Monax*). Two of these animals were given small doses of the oxime, mixed with a diet of cornmeal. The urine, passed in twenty-five and thirty-three hours respectively by the two chucks, gave a good indo-phenol test. Absorption doubtless took place in less than twenty-four hours.

Experiment VIII. Absorption in guinea pigs and white rats. Three guinea pigs were fed the oxime and killed at end of two, four and seven hours respectively. The urine gave the indo-phenol test in the four and seven hour pigs.

The experiments upon white rats were unsatisfactory.

2. *Absorption from subcutaneous tissue.* Thirty cubic centimeters of a saturated solution of the oxime in 0.9 per cent NaCl + 0.2 per cent Na_2CO_3 were injected subcutaneously into an etherized cat. Urine was withdrawn from the bladder by catheter at five minute intervals. The indo-phenol reaction was faintly shown five minutes after the injection, and at end of fifteen minutes it was very distinct. At end of forty-five minutes the urine took on a distinct lemon yellow color, showing unchanged oxime. At end of one hour and fifteen minutes the injection was repeated. There was an increased flow of urine, which became cloudy, and showed presence of a considerable amount of albumin, numerous granular casts, and some leucocytes and red cells. Cat died twenty-one hours after second injection. Much albumin found in urine. Bile was almost colorless and gave the indo-phenol test after boiling with H_2SO_4 . (It also contained red and white corpuscles.) Could not get the indo-phenol test in the liver, but it was positive in the mucosa of small intestine and in the contents of the large intestine.

The experiment was repeated several times on cats, rabbits and woodchucks, with the same results, except that no albuminuria resulted when quantities of 10 cc. or less were injected. In each case the drug was quickly absorbed and both the urine and bile gave the indo-phenol test.

A few experiments were made showing that absorption from the peritoneal cavity occurs rapidly also.

3. *Intravenous injection of the oxime, with blood pressure experiments.* **Experiment I.** A medium sized cat received, under ether, 35 cc. of a saturated solution of the oxime in the alkaline saline medium, in the course of forty-five minutes, by femoral vein. It was then killed by bleeding, and the blood, liver; intestinal mucosa, kidney and urine examined. p-amino-phenol was found in the urine in conjugated form, and also in the kidney and

mucosa of small intestine. Free p-amino-phenol was found in the blood. Unchanged oxime was found in the kidney urine and intestinal mucosa, but not in the liver. The urine was albuminous.

Experiment II. A large cat received 55 cc. of the above solution in one hour and one half. Conjugated p-amino-phenol was found in the liver, but not in the kidney and urine. It was found free in the blood. The oxime was found in the kidney only.

Samples of liver and kidney were removed for histological examination.

Experiment III. A full grown cat received 70 cc. of the solution in the course of one hour and thirty-six minutes. Conjugated p-amino-phenol was found in the liver, and unchanged oxime in the urine, which was not albuminous. Portions of the liver and kidney were removed for microscopical examination.

Experiment IV. In this experiment the blood pressure and urine flow were recorded. A large dog was used. 20 cc. of the saturated alkaline solution were injected by femoral vein, a total of 60 cc. being given. There was no effect upon blood pressure, and no increased flow of urine, except that due to the alkaline solvent used.

Experiment V. Another dog was used the blood pressure only being measured. 55 cc. of the oxime solution, injected into the femoral vein, failed to cause any rise in arterial pressure, though the heart beat became more rapid.

Experiment VI. The carotid pressure was recorded in four cats. Each received from 10 to 20 cc. of the oxime solution by femoral vein. In each cat there was a marked rise in blood pressure, varying from a minimum of 14 mm. in one cat to a maximum of 34 mm. in another. The rise came on quickly and in some cases lasted as long as a half hour. That this effect was due to the oxime was shown by control injections of the solvent, which alone caused no such increase in blood pressure.

ANTIPYRETIC ACTION

In view of the ready decomposition of the oxime, with p-amino-phenol as one of the products, it was easy to predict that the

substance would have antipyretic properties, providing it could be introduced into the system in sufficient amount. Its slow absorption in cats and dogs rendered them useless for experiments along this line. Accordingly, rabbits were used, and for the additional reason that fever can be readily induced in them.

Experiment I. A rabbit, weight 1.5 kg., was given 5 cc. of a 20 per cent solution of albumose hypodermically. At the end of the sixth hour thereafter the temperature had risen from 102.2

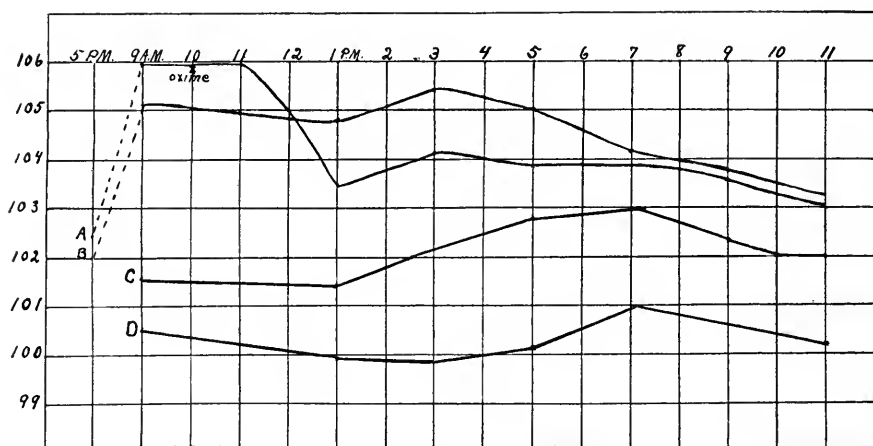


Fig. 1.

Fig. I. Effect of phenolphthalein oxime upon pyrexia in the rabbit. In rabbits A and B fever was produced by the subcutaneous injection of 5 cc. of 20 per cent albumose solution, at 5 p.m. The next day at 10 a.m. rabbit A received 0.5 gram of the oxime by mouth, and at 2.30 p.m. the dose was repeated. B received none. Rabbits C and D were control animals with normal temperatures. The chart shows a marked reduction of the temperature of rabbit A, which received the oxime.

to 104.2°. At this point 0.4 gram of the oxime was given in water per os. The temperature continued to rise and reached a maximum of 105.4° six hours after the oxime was given. A control rabbit, which received the same amount of albumose, showed a similar rise in temperature but in this control the temperature fell to normal less rapidly than it did in the oxime rabbit. The evidence of antipyretic action in the experiment was slight.

Experiments II to V. Four rabbits weighing from 1.3 to 1.5 kilos, were given by mouth oxime in amounts from 0.5 gram to 1.5 grams. They received the drug about twelve hours after the albumose was injected, and when the temperature was at its maximum point. Controls of normal rabbits and some with fever, produced as stated, were kept, and all were observed under a room temperature which did not vary over 2°C . After administration of the oxime to the four rabbits the temperature of

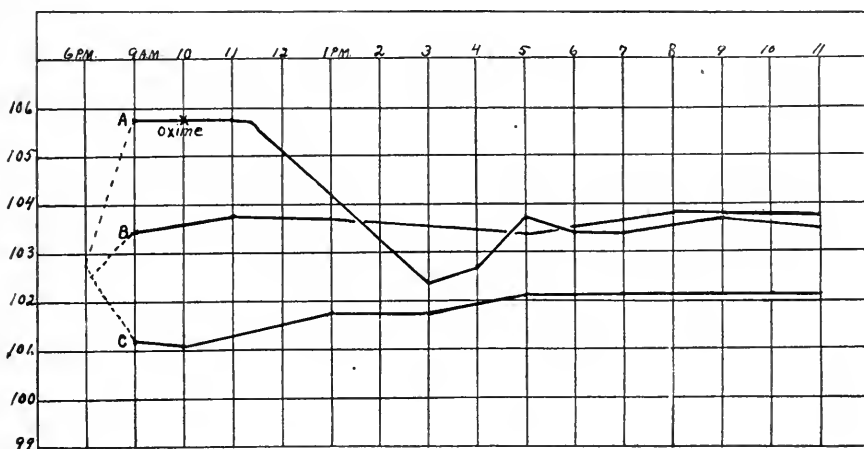


Fig. 2.

Fig. 2. This chart illustrates another typical experiment of this sort. Rabbits A and B received albumose as described above, at 6 p.m. At 10 a.m. the next day A received by mouth 0.7 gram of the oxime. B received none. C is a control rabbit with normal temperature.

each began to fall, in two cases within an hour, and in two others within $1\frac{1}{2}$ hours. The amount of fall ranged from $1\frac{1}{2}$ to 3°F . (temperature was taken in rectum in all cases). The lowest point was reached within two to five hours after administration of the oxime. Two charts are given below showing the course of the temperature in two of the rabbits.

It is seen that the oxime produces a marked fall in temperature in rabbits having fever produced as described. The early antipyretic effect coincides with the rapid absorption of the drug and the sudden, pronounced drop in some of the rabbits indi-

cates rapid decomposition of the oxime. The p-amino-phenol which is set free thus rapidly has a chance to exercise its toxic effects as well as its antipyretic action. For this reason the oxime has no value as an antipyretic.

TOXICITY OF THE OXIME

It was noticed that some of the rabbits used in the experiments upon absorption and reduction of temperature lost weight and in some cases died within a month or six weeks. In view of the well known cytolytic action of p-amino-phenol, the blood, liver, and kidneys, were examined histologically in three rabbits which had received 0.5 to 0.7 gram doses of the oxime. In these rabbits there was positive evidence of hemolysis, and the liver of one, which died five weeks after receiving the oxime, showed unmistakable signs of cell destruction. There was intense congestion of the organ. The kidneys in all three rabbits showed marked destructive effects, the cell protoplasm being dissolved completely in some localities. These changes were confined mostly to the medullary tubules and collecting tubules of the cortex, the convoluted tubules being affected to a much less extent.

In cats similar changes were noted in the kidneys and liver only after intravenous and hypodermic injection. These effects were compared with those produced by an intravenous injection of pure p-amino-phenol, an amount of the latter equivalent to that split off from the oxime being given, dissolved in 0.9 per cent NaCl solution. The cytolysis was not so extensive when the oxime was used, but was of the same character and was most marked in the kidney. With the p-amino-phenol, on the other hand, the cell destruction seemed to be most extensive in the liver. This interesting point needs further study and confirmation.

In these experiments with the intravenous injection of the oxime, there is one serious objection to the method. The weak Na_2CO_3 solution used has cytolytic properties itself, and it is difficult to say just how much of the cell destruction observed was due to the oxime alone. For that reason only a few experiments were made.

FATE AND ELIMINATION

This subject has already been considered in connection with the antipyretic action and toxicity of the drug, and but little more can be added at present. In the cat and dog most of the oxime is excreted unchanged with the feces. After absorption it meets the same fate in all animals, that is, it undergoes hydrolytic decomposition, p-amino-phenol being a constant product. While this last statement is not based upon direct chemical analysis of urine to determine the presence of free or combined p-amino-phenol, there can be no doubt that it was liberated from the oxime. This view is supported by (1) the indo-phenol test; (2) by the antipyretic action described. In regard to the indo-phenol reaction it might be claimed that, as is well known, other substances, as chinonchlorimid, could be responsible for the test, instead of p-amino-phenol. In reply to such a claim it may be said that there is no known reaction of this oxime which would yield any other substance giving the indo-phenol test except p-amino-phenol. The experiments of this research indicate consistently that this oxime undergoes the same hydrolytic cleavage in the body that it does in the test tube.

Professor Orndorff has suggested that, like other oximes, this one may decompose, on standing in an alkaline solution, yielding hydroxamic acid. In such a case the same sort of decomposition might be possible in the body. Examination shows, however, that this oxime does not undergo such spontaneous change in vitro, and the surmise is that it does not occur in vivo.

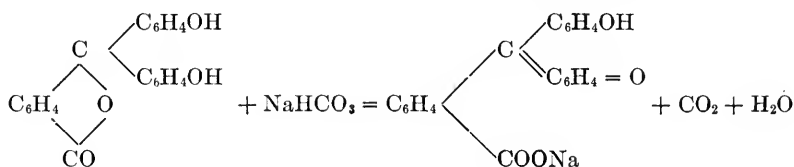
That this oxime does not split up so as to liberate phenolphthalein itself was shown by the failure of the urine to give a test for phenolphthalein.

Mention has been made of a diuretic effect following the administration of this oxime to rabbits. This occurred only where large doses were given, and was doubtless due to the irritant action of the p-amino-phenol. In the dog an experiment was made on diuresis, the oxime being given in alkaline solution by vein in large amounts. No increased flow of urine was obtained, except that due to the fluid injected.

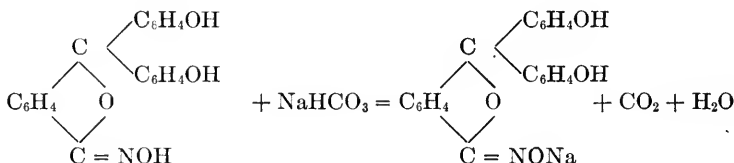
DISCUSSION OF RESULTS

From the data detailed in the preceding pages four facts of importance in regard to the physiological action of phenolphthalein oxime may be reviewed. They are (a) the absence of purgative action; (b) absorption from the intestine; (c) antipyretic action; (d) fate in the organism, and toxic effects.

(a). *Lack of purgative action.* While no complete explanation can be given for the failure of this derivative of phenolphthalein to induce at least a mild laxative effect, no doubt the result is to be traced, as Professor Orndorff believes, to the chemical structure of the compound. In order to make this view clearer we may compare a reaction of phenolphthalein with a similar one in the case of the oxime. When the former substance enters the intestine the following chemical reaction takes place:



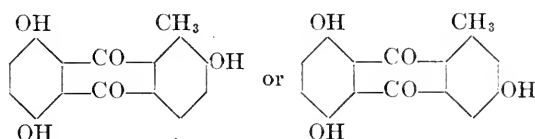
This is the ordinary reaction which occurs when phenolphthalein comes in contact with an alkaline carbonate, a red salt resulting. On the other hand, a substance of different structure, and yellow in color, is formed when the *oxime* of phenolphthalein reacts with an alkaline carbonate. The chemical change which would occur in this case may be written thus:



The exact mechanism of the laxative action of phenolphthalein itself is not understood, except that it acts upon some structure of the intestinal wall, probably the mucosa and reflex mechanism of peristalsis, producing a loose, watery stool, together with increased muscular contraction. At this point may be stated

some of the views held regarding the purgative action of certain important drugs, giving especial attention to phenolphthalein and the oxime under consideration.

It will be noted that when phenolphthalein reacts to form the red salt it goes into the *quinoid* form, and in this form it undoubtedly exists in the intestines and body fluids. The *oxime* of phenolphthalein, on the other hand, does not take on the quinoid structure when it reacts with the alkaline carbonates. Other phenolphthalein derivatives which are purgative, such as the tetrachlor compound, have the quinoid structure in their salts, and it is noteworthy that the anthracene derivatives, which yield the active principles of the purgative drugs—Senna, Rhubarb, Aloes, and Cascara, have the quinoid, or quinone, arrangement. As an example may be mentioned trioxymethyl-anthraquinone, or emodin, which has the formula:⁵



In view of these facts, Professor Orndorff is strongly of the opinion that the purgative action of these various substances is associated in some way with the quinoid structure of the molecule. But the question is a complex one, and is much in need of further study. The most important published investigations along this line so far are those of Tschirch⁶ on the anthraquinone derivatives in Frangula, Senna, Rhubarb, and Aloes; the work of Brissemoret⁷ on the ketone groups and hydroxyl and alliphatic side chains; and on the synthetic side the work of Vieth⁸ on the importance of the position of the hydroxyl in the anthraquinones.

⁵ Meyer und Gottlieb, *Experimentelle Pharmacologie* 1910, 175.

⁶ Schweiz. Wochensh. f. chemie u. Pharmazie, nr. 23, 1898; Bd. 42, nr. 35, 1904; Ber. d. deutsch. pharm. Gesell., 174, 1898; Arch. der Pharmazie Bd. 237, 1899, Bd. 238, 1900; Pharm. Post. 17-19, 1904 das Lit.

⁷ Contrib. à l'étude des purgatifs organique. Thesis, Paris, 1903, Joamin and Co.; Bull. des Sci. pharmacol., 1903, T. vii, p. 17.

⁸ Münch. Med. Wochensh., 1901 nr. 35, s. 1381.

From these different investigations the purgative principles of these drugs have been variously attributed to the anthracene groups, to the ketone groups in anthraquinone, and to the presence of hydroxyl and aliphatic side chains. It is difficult to know to which view most importance should be attached. It is believed that the suggestion put forth in this paper on the possibility of the quinoid structure having some connection with the purgative action of the compounds discussed will be found of interest.

Returning to the non purgative action of phenolphthalein oxime, there are two other suggestions which may be offered on this point. The tendency of the oxime to undergo hydrolytic splitting, resulting in the formation of products which might be inert in the bowel, should not be forgotten. However, there is no evidence that such decomposition takes place, either before or during absorption, to any marked extent. A little p-amino-phenol may be found in the intestinal contents, but the most of that formed is quickly absorbed and excreted. Finally, the oxime might be absorbed either too rapidly or too slowly to have any effect. Experiments are given which support this possibility.

(b). *Absorption of the oxime.* What happens to the oxime during absorption cannot be said. There is no evidence at hand that much decomposition takes place in the mucosa. The experiments rather indicate that this occurs elsewhere in the body. The drug is taken up mainly by the small intestine. Why this occurs so slowly in the dog and cat is a question which must, at present, remain unanswered. Selective absorption is a phenomenon of which there are many examples, and the present case adds another. While this instance is not so remarkable as the selective absorption of the tetrachlor compound of phenolphthalein by the large intestine,⁹ it is none the less difficult to explain.

Absorption from subcutaneous tissue and from the peritoneal cavity does not call for any special comment.

⁹ Abel and Rowntree, *Loc. cit.*

(c) *Antipyretic action.* As already stated, the character of the decomposition which this oxime undergoes led to the experiments in this direction. While the number of experiments performed was small, there can be no doubt that a marked lowering of temperature occurred. But it will be noticed that rather large doses of the oxime were given. A trifle less than one-third of the molecular weight of the oxime is due to the p-amino-phenol in the molecule. From this fact it may be seen that the rabbits in which a fall in temperature took place received from 0.17 to 0.49 gram of p-amino-phenol, which is more than would be liberated from an efficient dose of acetanilid, for instance.

It is known that the antipyretic action of the p-amino-phenol derivatives is, within certain limits, proportional to the amount of p-amino-phenol (or acetyl amino-phenol) liberated in the body. The same law seems to hold good for the temperature reducing power of this oxime. 0.3 gram has no effect upon the febrile temperature in the rabbit. From 0.5 gram on, the amount of fall and the length of time the temperature remains down depend to a large extent upon the quantity of the oxime given, or, in other words, to the amount of p-amino-phenol liberated. The upper limit, at which no increase in the effect can be obtained, has not been determined, but it is probably about a 2 gram dose of the oxime for the rabbit.

(d). *Fate in the organism and toxic effects.* The evidence at hand bearing upon the fate of our drug in the body has been given. Further study of this matter is necessary before the subject is entirely cleared up. It has been thought best to make such a study the basis of a possible future research.

In view of the well known toxicity of p-amino-phenol and in view of the ease and rapidity with which it is split off from phenolphthalein oxime in the body, the toxic effects noted in the experiments upon rabbits and cats have been attributed to this amino-phenol. There remains the possibility, however, of the p-oxy-o-benzoyl benzoic acid being poisonous. While the experiments bearing upon the physiological action of this portion of the oxime are incomplete, the results so far are negative. The only effects following the administration of the oxime are typical

of those of p-amino-phenol, and the most plausible explanation of the occasional toxic action is, therefore, that in those animals in which the oxime is absorbed quickly the decomposition goes on so rapidly that the capacity of the body to conjugate the liberated p-amino-phenol is overwhelmed, cytotoxicity resulting. In this connection it would be of great interest to know where this decomposition and conjugation take place. While the writer has no experiments bearing upon this point directly, the extensive cell destruction in the liver and kidneys is suggestive. The same problem has arisen, of course, in connection with other antipyretics which liberate p-amino-phenol or other toxic product. It is one of great physiological and pharmacological interest and is yet awaiting solution.

SUMMARY

1. Phenolphthalein oxime readily undergoes hydrolysis, both in vitro and in vivo, yielding p-amino-phenol and p-oxy-o-benzoyl benzoic acid in each case. By virtue of this decomposition, it differs markedly from other phenolphthalein compounds in its physiological effects.

2. It has no local action on animals and does not retard the growth of bacteria.

3. It is devoid of laxative action. An explanation is offered for this result, namely, that it is due to the absence of the quinoid structure, which is present in other purgative phthaleins and in the anthraquinone purgatives.

4. It is not absorbed from the stomach, but is taken up quite rapidly from the small intestine in all animals experimented upon except the dog and cat, which excrete most of the drug by the feces.

5. The mechanism of absorption is not understood, but experiments indicate that it is not attended by decomposition of the oxime.

6. After absorption, hydrolysis of the oxime quickly takes place, in all animals, with the liberation of the products mentioned. The animal body is limited in its power to carry on this hydrolysis, unchanged oxime being eliminated by the kidneys in

those animals which absorb it quickly, as in the rabbit and woodchuck, and in other animals after large subcutaneous doses. A mild diuresis may follow in these instances, due to the irritant action of the liberated p-amino-phenol.¹⁰ The liver and kidney suffer from the cytolytic action of the amino-phenol, as do also the blood cells.

7. As a further result of the liberation of p-amino-phenol antipyretic effects may be obtained in those animals which absorb the oxime rapidly. The reduction in temperature is proportional to the amount of p-amino-phenol set free.

8. The p-oxy-o-benzoyl benzoic acid set free in this hydrolysis has been without physiological effect in these experiments. How it is eliminated has not been determined.

The writer wishes to express his indebtedness to Prof. W. R. Orndorff for valuable suggestions during this investigation, and for materials supplied from his laboratory. His thanks are also due to Prof. Sutherland Simpson for kindly criticisms.

¹⁰ And possibly also to unchanged oxime, in part.

ON THE CONVULSANT ACTION OF ACID FUCHSIN UPON FROGS DEPRIVED OF THEIR CARDIAC CIRCULATION

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I. INTRODUCTION

In communications which appeared recently from this laboratory,¹ facts were reported which definitely demonstrated that soluble substances may be satisfactorily distributed throughout the body of frogs, after complete elimination of the circulatory apparatus. This was shown by sharp reactions of some organs to substances which were introduced into the body of frogs after complete removal of the entire heart which, of course, meant also the elimination of the activity of the lymphatics and the lymph hearts. The chief requirement is that the organs giving the reactions should not die off too rapidly in consequence of the complete anemia. The iris and the spinal cord were employed as reacting organs, and as reagents were used adrenalin, which caused a definite dilatation of the pupils, and strychnin and morphin which caused paralysis and spasms. Since in these animals the central organ for circulation and distribution was eliminated it was assumed that the distribution was accomplished by way of the lymph spaces which intercommunicate, more or less freely throughout the entire body. In contrast to the centralizing cardio-vascular mechanism, the system of lymph spaces which are the agents of distribution, was designated, as *peripheral mechanism*. The action of morphin brought out an especially

¹ Meltzer: Jour. of Exper. Medicine, xiii, 542, 1911.

striking result. While in a normal frog an injection of, say 10 or 15 mgms. of morphin, causes at first no effect at all, but may cause spasms a few days later, in a cardiectomized frog, 7 or 8 mgms. would cause spasms and tetanus within 40 or 50 minutes after the injection. The interpretation offered for this paradoxical phenomenon was, that the blood of the normal circulation may contain substances, taken up from some secretory organs, which are capable of neutralizing and delaying the spasmodic action of morphin; which substances are lacking when the morphin is carried to the central nervous system by the peripheral mechanism.

With the special interest which naturally attaches itself to this unusual action of morphin under the given circumstances, it was desirable to discover other substances which might act in a similar manner. After the appearance of the interesting communication of Barbour and Abel² on the action of acid fuchsin upon frogs, we believed we had reason for the assumption, that fuchsin might prove to be another such substance. Barbour and Abel found, in the first place, that acid fuchsin, which hitherto was considered to be non-toxic, may bring out in normal animals, by injection into a lymph sac, a typical series of convulsions. However, an analysis of numerous experiments on frogs has shown to the authors "that tetanus will not appear, as a rule, after fairly large doses (1 to 4 mgrs. per gram body-weight of the drug) until from 1 to 20 hours or more have elapsed. In a certain number of cases no effect whatever will be produced by doses of this size or by a much larger dose." This behavior of the action of fuchsin recalls to mind the action of morphin upon normal frogs. Furthermore, Barbour and Abel discovered, that after the removal of the anterior third of the cerebrum of the frog, injections of fuchsin bring out convulsions with much greater readiness, sometimes in less than one minute, and with much smaller doses, in one instance with a dose of only 0.35 mgm. per gram of body-weight. Here was a fact which demonstrated that under certain circumstances fuchsin, like morphin, might act much more

² Barbour and Abel: *The Jour. of Pharmacol. and Experiment. Thera.*, ii, 167 (1910).

readily than in normal frogs. We have, therefore, with the consent of Professor Abel, carried out a series of experiments in which the effect of injections of fuchsin was studied upon cardiectomized frogs.

II. EXPERIMENTAL RESULTS

Methods

Acid fuchsin was used at first, as in the experiments of Barbour and Abel, in a 5 per cent solution. Later, when very small doses of the fuchsin were administered much higher dilutions had to be employed. In the earlier experiments aqueous solutions were used; later, however, in order to avoid the irritating action of the distilled water, the fuchsin was dissolved in a solution of 0.6 per cent NaCl, the "physiological salt solution" for frog tissue. The injections, when not stated otherwise, were given into the dorsal lymph sac. Practically all experiments were made on the species *Rana pipiens*. We had no definite information, from what region in the country the frogs came and when they were caught. The experiments were carried out in the months of April and May. All the animals were *etherized during the operation* and the ether removed immediately on closing the wound; they were under ether not longer than 5 to 6 minutes. The cardiectomy was performed by first ligating the heart as high above the auricle as possible, whereupon ventricle and auricles were cut off with scissors. In most of the experiments very little blood was lost in the operation. Immediately after closing the abdominal wound the animals were taken off the board and put in a normal position. The injections were made, generally, between 2 to 6 minutes after removing the ether, from which, by this time, the animals had fairly recovered.

We made very few control experiments, that is, we made no special study of the action of fuchsin on perfectly normal animals, since this was extensively done and well described by Barbour and Abel.

Observations and Results

The course of the series of phenomena which we observed following the injection of fuchsin was practically the same as was described by Barbour and Abel. It began first, at least often, with symptoms which are to be characterized as depression, then followed a series of manifestations of hyperexcitation, beginning with spontaneous spasms in which the flexors had a predominant share, and finishing up by a more or less complete extensor tetanus of the strychnin type. At a later stage convulsions or twitches came only in response to tapping; depression and death closed the scene. We shall disregard here the details of the phenomena of depression as in the absence of cardiac circulation it is difficult to decide, how much of the depression may be due simply to the final paralysing effect of the anemia. *The excitation phenomena, however, were entirely due to the drug action, as cardiectomized frogs never show any signs of excitation.* We shall not lay too much stress either upon the character and the duration of the periods of the spasms; the effect of the increasing room temperature in the months during which the experiments were performed is here a confusing factor. The onset of unmistakable convulsions is the phenomenon which is of chief interest in our present investigation.

We began our experiments at first with injections of doses of fuchsin which were larger than 1 mgm. per gram of body-weight but reduced the dose soon to 1 mgm. fuchsin per gram frog. Seven experiments were made with this dose. Then the dose was further reduced to 1/2, 1/4 and 1/10 of a milligram per gram body-weight; with which doses all together only nine experiments were made. All these experiments have shown unmistakably the effective action of fuchsin upon the cardiectomized animals. It is, however, unnecessary to give here any details of these experiments, nor to present them in a table. The results were just as striking and convincing in the experiments in which the acid fuchsin was administered in doses of only 1/20 of a milligram per gram frog. This series consisted of twenty-two experiments (see table I) which we shall now report somewhat in detail. In all

these experiments a 1 per cent solution of fuchsin in "physiological" salt solution (0.6 per cent NaCl) was used. We shall illustrate the results by a few abbreviated protocols of the experiments.

Experiment 1. April 14, 1911. Frog no. 25, male, 29 grams.

- 1:53 Heart removed, moderate loss of blood.
- 1:59 Injected into dorsal lymph sac $1/20$ mgm. (per gram frog) of 1 per cent acid fuchsin in frog saline.
- 2:03 Strong, spontaneous *flexor* convulsion.
- 2:04 Another powerful convulsion.
- 2:09 Still has powerful flexor convulsions, one right after another, (probably 15 or 20 convulsions already), throws himself about violently, sometimes turning over and over.
- 2:12 Has begun to show strong *extensor* convulsions now.
- 2:17 Still has occasional extensor convulsions; gradually becoming less frequent; lies extended all the time.
- 2:27 Has had no spontaneous convulsions for about seven minutes; a light tap gives a transient but good extensor tetanus.
- 2:37 A light tap gives good twitch.
- 3:23 A strong tap gives faint twitch.
- 4:55 Repeated strong tapping gives a faint response only.

Experiment 2. April 14, 1911, frog 26, male, 35 grams.

- 2:21 Heart removed.
- 2:25 Injected into dorsal lymph sac $1/20$ mgm. (per gram frog) of 1 per cent acid fuchsin in frog saline.
- 2:33 Strong, spontaneous *flexor* convulsions.
- 2:36 Already has had several powerful flexor convulsions; flops about table violently.
- 2:38 Beginning *extensor* convulsions.
- 2:42 Has two or three spontaneous, strong extensor convulsions per minute.
- 2:48 Still has powerful, rapidly repeated, spontaneous convulsions.
- 2:52 *Spontaneous convulsions have ceased since 2:50*; a slight touch elicits strong extensor convulsions.
- 3:23 Strong repeated tapping gives slight twitch.

Experiment 3. April 18, frog 35, male, 22 grams

- 1:29 Heart removed, no loss of blood.
- 1:32 Injected into dorsal lymph sac 1/20 mgm. per gram bodyweight of 1 per cent acid fuchsin in frog saline.
- 1:44 First spontaneous strong *flexor* convulsion.
- 1:46 A powerful, spontaneous, flexor convulsion quickly followed by several other exceedingly strong ones.
- 1:49 Powerful flexor convulsions follow each other rapidly.
- 1:54 Still shows very strong flexor convulsions, but less frequently.
- 1:59 Shows spontaneous *extensor* convulsions.
- 2:09 No more spontaneous convulsions.
- 2:19 A light tap gives good extensor tetanus.
- 2:35 Repeated tapping gives good twitch.
- 2:45 No response at all.

Experiment 4. April 11, frog 22, male, 22 grams

- 1:14 Heart removed, no blood lost.
- 1:19 Injected into dorsal lymph sac 1/20 mgm. per gram bodyweight of 1 per cent fuchsin in frog saline.
- 1:45 Powerful spontaneous flexor convulsions, one convulsion after another; writhing about.
- 1:57 Still has powerful convulsions, mixed type now, *flexor* and *extensor* (roof of mouth very red).
- 1:58 Strong *extensor* convulsions.
- 2:04 Still has spontaneous, powerful, sustained extensor convulsions, occasionally there is strong abduction of thighs and dorsal flexion.
- 2:20 No spontaneous convulsion for ten minutes; a light tap gives strong, sustained extensor convulsion.
- 2:34 Repeated tapping gives good but transient tetanus.
- 3:30 Repeated tapping still gives fair twitch.
- 3:39 Strong repeated tapping gives only slight response.
- 3:45 No response at all.

Experiment 5. April 18, frog 37, male, 23 grams

- 1:54 Heart removed, moderate loss of blood.
- 1:57 Injected into dorsal lymph sac 1/20 mgm. (per gram frog) of 1 per cent fuchsin in frog saline.

- 2:14 First spontaneous *flexor* convulsion.
2:22 Since 2:16 has shown no convulsion, definite depression.
2:27 Is having now repeated, spontaneous, strong *flexor* convulsions.
2:52 No spontaneous convulsions for about five minutes. Tapping gives strong, sustained convulsions, mixed *flexor* and *extensor* type.
3:01 Mild tapping gives strong sustained *extensor* convulsions.
3:32 Repeated tapping gives slight twitch.
3:42 Very faint response.

These five protocols represent fairly well the varieties of behavior of the twenty-two cardiectomized frogs (table I) which received $1/20$ mgm. per gram fuchsin into the dorsal lymph sac. Without exception they all responded, sooner or later, with spontaneous violent convulsions. The *flexor* type appeared first. In most cases also spontaneous, sustained, *extensor* convulsions followed which, as a rule, did not last long. However, touching or tapping practically never failed to bring out for some time a sustained *extensor* tetanus, even after the spontaneous convulsions had ceased. Between the convulsions, the frogs were, as a rule, deeply depressed. The shortest interval between the injection of fuchsin and the appearance of a *flexor* convulsion, was in this series 4 minutes and the longest 27 minutes.

This entire series of experiments was carried out in the month of April.

Two experiments were made with $1/30$ mgm. (per gram frog), and two with $1/40$ mgm. (per gram frog) of 1 per cent acid fuchsin in frog saline, injected into the dorsal lymph sac. The entire quantity injected was not much more than 0.1 cc. All four frogs developed convulsions but their entire course was much weaker than with the dose of $1/20$ mgm. per gram frog. In one frog ($1/30$ mgm. per gram frog) the first sign of a convulsion appeared 51 minutes after injection, and the spasms were very weak. The following is the protocol of the one of these four experiments in which the effects were strongest.

TABLE I

Injections of 1 per cent acid fuchsin, in frog saline, into the dorsal lymph sac of cardiotomized frogs to determine the minimal convulsant dose of fuchsin. Table shows time in which convulsions developed and their duration.

NUMBER OF EXPERIMENT	WEIGHT	DOSE PER GRAM FROG	TIME FROM OPERATION TO INJECTION	TIME FROM INJECTION TO FIRST CONVULSION	DURATION OF FLEXOR CONVULSION	DURATION OF EXTENSOR CONVULSION	TIME FROM INJECTION TO LOSS OF IRRITABILITY
	<i>gms.</i>	<i>mgm.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>
16	39	1/20	4	11	14	22	139
22	32	1/20	5	26	13	36	146
24	31	1/20	3	27	23		108
25	29	1/20	6	4	9	15	176
26	35	1/20	4	8	5	14	80
27	28	1/20	3	13	9	14	121
28	35	1/20	5	9			120+
30	25	1/20	3	15	39		81
32	29	1/20	3	22	31		112
33	30	1/20	3	15	21	40	113
34	28	1/20	3	22	37		89+
35	22	1/20	3	12	15	20+	73
36	27	1/20	4	6	61	22	120
37	23	1/20	3	17	45	12	105
38	29	1/20	5	18	44	21	91
39	20	1/20	5	12	28	22	
40	27	1/20	3	27	11		
41	25	1/20	3	24	11+		
47	31	1/20	5	12	24	31	134
48	24	1/20	3	16	13+		76
49	28	1/20	3	18			132
50	24	1/20	3	10	26	16	99
29	38	1/30	3	51	17		148
31	30	1/30	3	19	30		102
17	40	1/40	4	26	2	25	68
23	29	1/40	3	49	18		97+

Experiment 6. April 6, frog 17, female, 40 grams

- 2:33 Heart removed, no bleeding.
 2:37 Injected into dorsal lymph sac 1/40 mgm. (per gram frog) of 1 per cent acid fuchsin in frog saline.
 3:03 Shows first *flexor* convulsion.
 3:05 Shows spontaneous, strong, extensor convulsions.

- 3:14 Has shown three spontaneous convulsions. Lies extended all the time; a light tap gives a good sustained tetanus, but it is not violent; does not throw itself about table.
- 3:30 No spontaneous convulsions since last note; responds to moderate tap with good transient tetanus.
- 3:45 No response at all.

No attempt was made to establish the effect of still smaller doses of fuchsin by the lymph sac injection method. The minimal effective dose of acid fuchsin for cardiectomized frogs is probably not much lower than 1/40 mgm. per gram frog.

Etherization. From the experiments of Barbour and Abel it was not evident that their frogs were etherized shortly before the injection; neither was there any reason for such a procedure in their experiments. Our animals, however, were in every case under ether, for a few minutes, during the operation for the removal of the heart. In order to establish whether the etherization was a factor in the subsequent appearance of the convulsions, we have etherized frogs for about 8 minutes without removing their hearts, or afflicting any other injury, and a few minutes later injected into the dorsal lymph sac 1/10 mgm. (per gram frog) acid fuchsin in frog saline. Five frogs were treated in this manner and none showed any reaction whatsoever during the several days that they were under observation.

The experiments reported have confirmed, by another method, the statement of Barbour and Abel concerning the considerable latent toxicity of acid fuchsin. They have further demonstrated again that a soluble substance can be efficiently transported from one part of the body to another by the exclusive aid of the *peripheral mechanism*. After injection of a very small quantity of acid fuchsin into the dorsal lymph sac, the roof of the mouth became red in a very short time, and symptoms set in which indicated the presence of fuchsin at some part of the central nervous system. *They have, finally, brought forward another striking and important instance of a substance which can be greatly more effective when distributed within the body exclusively by way of the peripheral lymph space mechanism than by the otherwise very efficient central cardio-vascular mechanism.* In normal

frogs after injections of acid fuchsin, "a tetanus will not appear, as a rule, after fairly large doses (1 to 4 mgm. per gram of body-weight of the drug) until from 1 to 20 or more hours have elapsed" (Barbour and Abel). In our experiments 1/20 mgm. of fuchsin per gram body-weight invariably brought out violent convulsions in intervals of much less than one hour. *Acid fuchsin, then, is at the very least, twenty times more toxic when distributed by the peripheral mechanism, than when distributed in the normal animal by the central circulation. Furthermore the drug proved to be here even more toxic than for frogs whose anterior part of the cerebrum was removed.* According to Barbour and Abel, 0.35 mgm. of fuchsin per gram body-weight was the minimal toxic dose for decerebrated animals. Assuming that this was a reliable minimum dose (in the protocols and tables of Barbour and Abel there are not many instances in which 0.35 mgm. per gram frog proved to be a reliably toxic dose) it is still *seven times as large* as the infallibly convulsant dose in cardiectomized frogs.

There is, however, one circumstance by which the toxicity of fuchsin would appear to be greater in decerebrated than in cardiectomized frogs. In the experiments of Barbour and Abel on frogs without the anterior part of the cerebrum the convulsions sometimes appeared in less than one minute after the injection of fuchsin. While this may not have been a frequent occurrence, it remains nevertheless an indisputable fact that the average length of the interval in the mentioned experiments of Barbour and Abel was definitely shorter than the average interval in our series of experiments with 1/20 mgm. of fuchsin per gram frog. It must be remembered, however, that in the experiments of Barbour and Abel the cardio-vascular mechanism remained intact and that the transportation of the fuchsin was accomplished essentially by this mechanism. Now, whatever we may claim for the efficiency of the peripheral lymph space mechanism, it must remain undisputed that the rapidity of the transportation will always be greater when accomplished by the cardiac circulation. In other words we surmised that the shortness of the interval was essentially due to the aid of the intact circulation, and not to a special virtue of the removal of the anterior part of the cerebrum. To

the weight of this plausible reasoning we tried to add the test by experimentation. In five experiments the removal of the anterior part of the brain was added to the cardiectomy and then 1/20 mgm. (per gram body-weight) of the fuchsin was injected. If the shortness of the interval in the brain experiments of Barbour and Abel were due not to the cardiac action but to the removal of the anterior part of the brain, the interval in these experiments ought to be definitely shorter. The results of these experiments are incorporated in table II. *The convulsions in these experiments did not set in earlier than in cardiectomized frogs*

TABLE II

In these experiments the frogs were cardiectomized and in addition the anterior one-third of the cerebrum was removed. Fuchsin injected into dorsal lymph sac—using 1 per cent solution in frog saline.

NUMBER OF EXPERIMENT	WEIGHT	DOSE PER GRAM FROG	TIME FROM OPERATION TO INJECTION	TIME FROM INJECTION TO FIRST CONVULSION	DURATION OF FLEXOR CONVULSION	DURATION OF EXTENSOR CONVULSION	TIME FROM INJECTION TO LOSS OF IRRITABILITY
	<i>gms.</i>	<i>mgm.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>
54	62	1/20	4	55			216
55	52	1/20	2	30+			96
56	52	1/20	3	32	56	60	213
57	55	1/20	3	19	50	40	139
58	51	1/20	3	20	74	40	189
59	60	1/20	6	11	9	44	87

with intact brains; the procedure of the removal of the anterior part of the brain, therefore, certainly did not accelerate the development of the convulsions. On the contrary, the experiments seem to indicate even an opposite result. In one experiment the onset of the convulsions was very late and their course surprisingly weak; we reproduce here its protocol.

Experiment 7. May 3, frog 35, male, 52 grams

10:48 *Heart removed, not a drop of blood lost.*

10:50 *Injected into dorsal lymph sac 1/20 mgm. per gram of body-weight of 1 per cent fuchsin in frog saline.*

- 10:51 *Anterior part of brain snipped off with scissors, cut just back of eyes; very little loss of blood.*
- 11:50 Has had no spontaneous convulsions, but for some time responds to tapping with a good flexor convulsion. Sits on its haunches with head up.
- 12:15 No spontaneous convulsion yet; tapping gives a fairly good flexor convulsion.
- 12:26 Tapping brings almost no response.

This experiment presents a surprise; in the experiments with cardiectomy alone no such a weak response was ever met with after an injection of 1/20 mgm. per gram body-weight. Besides, the average interval between the injection and the first appearance of convulsions is for these five experiments much longer than the average interval for the frogs with cardiectomy alone.

However, we shall not enter in this paper upon a discussion of the possible significance of this apparently paradoxical fact. The number of these experiments is too small anyhow to serve as a basis for a proper discussion.

III. INTRA-VASCULAR INJECTIONS OF FUCHSIN

Intra-aortic Injections

The method of experimentation employed in the foregoing investigation was devised, as stated at the beginning, to study two questions; the distribution of the fuchsin by the peripheral mechanism and the favorable effect of the absence of the neutralizing blood upon the action of that drug. The question of the rapidity of action could not be well studied, as indicated before, in the absence of the cardiac mechanism. In the following series of experiments, we neglected the action of the peripheral mechanism and tried instead to study the action of fuchsin under a combination of the factors of absence of blood and rapidity of administration of the drug. For this purpose a cannula was tied into the *bulbus arteriosus* and the drug injected directly into the common aorta. The solution was thus rapidly distributed through the arterial system, in a manner equal to the distribution

by the pumping of the heart. (The cannula was filled with frog's saline, the attached piece of rubber tubing clamped, and the injection made with a fine hypodermic needle which was inserted through the rubber tubing). In some instances the heart was first cut off and the animal bled, in others the heart was clamped off, so as to avoid bleeding. The main purpose of the arrangement was, as stated before, to bring the drug rapidly to the nervous system, while, at the same time preventing the entrance of fresh blood during the injection of the drug as well as later.

At the beginning of these new experiments doses of 1/20 mgm. fuchsin per gram frog were employed. This dose, however, was soon reduced to 1/100 mgm., and less, per gram frog. In the latter cases the fuchsin had to be administered in still more dilute solutions, usually 1/10 per cent in frog saline. Although these experiments were made in the month of May with a room temperature often at 26° or 27° C. at which the cardiectomized frogs usually did not last long and did not respond readily with convulsions, the results were nevertheless very striking. We shall illustrate them with a few short protocols.

Experiment 8. May 4, frog 60, female, 72 grams

- 1:15 Bled by cutting off tip of ventricle, the cannula inserted into *bulbus arteriosus*.
- 1:40 Injected into cannula 1/20 mgm. (per gram frog) of 1 per cent fuchsin in frog saline; followed by washing the cannula with some saline. *Before the procedure could be finished the animal was having powerful convulsions.*
- 1:45 Is stretched out in continuous *extensor* convulsions.
- 1:52 Convulsions are weaker.
- 2:12 For a long time no spontaneous convulsions; a tap gives a good, but very transient tetanus.
- 2:30 No response.

Experiment 9. May 4, frog 61, female, 78 grams.

- 2:20 Bled, cannula inserted in *bulbus aortae*.
- 2:27 Injected 1/100 mgm. (per gram frog) of 1/10 per cent fuchsin in frog saline.

- 2:29 Spontaneous powerful *flexor* convulsions.
- 2:31 Since 2:29 has had continually powerful flexor convulsions.
- 2:32 Most powerful continuous *extensor* convulsions.
- 2:36 Only occasionally spontaneous convulsions; a light tap gives very strong extensor convulsions.
- 2:44 Still shows occasionally a transient extensor convulsion.
- 3:15 No response at all.

Experiment 10. May 8, frog 70, male, 61 grams

- 3:22 Cannula in bulbus aortae, no bleeding.
- 3:29 Injected $1/125$ mgm. (per gram frog) of 1 per cent fuchsin in frog saline.
- 3:35 A powerful spontaneous *flexor* convulsion, animal writhed and flopped about table.
- 3:37 One powerful convulsion after another.
- 3:44 The first *extensor* convulsion, spontaneous, strong.
- 3:49 Still shows spontaneous extensor convulsions; less frequently now.
- 3:54 Repeated tapping gives no response.

Experiment 11. May 8, frog 69, male, 76 grams

- 3:05 Cannula in bulbus aortae, no bleeding.
- 3:14 Injected $1/150$ mgm. (per gram frog) of $1/10$ per cent fuchsin in frog saline.
- 3:30 No convulsion yet.
- 3:32 A light tap gives a moderate convulsion.
- 3:33 The first spontaneous convulsion, moderately strong.
- 3:37 Has had three or four spontaneous moderately strong convulsions.
- 3:43 Repeated tapping brings no response.

Experiment 12. May 5, frog 65, male, 56 grams

- 1:40 Cannula in bulbus, moderate bleeding.
- 1:49 Injected $1/200$ mgm. (per gram frog) of $1/10$ per cent fuchsin in frog saline.
- 2:01 No convulsions, still jumps about.
- 2:09 Depressed, nose on table, tapping elicits no convulsions.
- 2:29 Strong depression. Tapping gives almost no response of any kind.

When injected directly into the aorta a dose of 1/20 mgm. per gram frog brought on most violent convulsions in less than a minute. The convulsions were very violent also with much smaller doses of the drug, but *the smaller the dose the later the convulsions set in*. However, at the utmost, it was only a question of a few minutes. A dose of 1/125 mgm. per gram frog was *the reliable minimum*; 1/150 mgm. per gram frog had only a very mild and unreliable effect; 1/200 mgm. per gram frog seems to cause no increase of excitability. (The experiments with 1/200 per gram frog demonstrate at the same time, that the injection of saline has no share in the production of convulsions.) See table III.

Intravenous Injection

These experiments proved, then, unmistakably the fact that by direct, intra-aortic injections, in the absence of continual

TABLE III

Intra aortic injections of $\frac{1}{10}$ per cent acid fuchsin in frog saline solution, in cardiectomized frogs.

NUMBER OF EXPERIMENT	WEIGHT	DOSE PER GRAM FROG	TIME FROM OPERATION TO INJECTION	TIME FROM INJECTION TO FIRST CONVULSION	DURATION OF FLEXOR CONVULSION	DURATION OF EXTENSOR CONVULSION	TIME FROM INJECTION TO LOSS OF IRRITABILITY
	<i>gms.</i>	<i>mgm.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>
60	72	1/20		1	4—	27	50
61	78	1/100	7	2	3	12	48
64	76	1/100	8	4	3	11	31
70	61	1/125	7	6	9	5	25
71	68	1/125	8	7			31
72	61	1/125	9	8	4	17	34
89	54	1/125		2			18
90	52	1/125	2	2	5	4(?)	13
91	56	1/125		6			27
93	50	1/125		3			21
96	72	1/125	All response lost in 25 min.				
68	58	1/150	11	17	-----8-----		30
69	76	1/150	9	18	-----6-----		30
63	91	1/200	No convulsions.				
65	56	1/200	9	No convulsions.			
66	59	1/200	10	No convulsions.			
67	93	1/200	8	No convulsions.			

entrance of fresh blood from all parts of the body, fuchsin, even in minute doses, will bring on violent convulsions in a very short time after the injection. The question, however, might arise whether the absence of the blood is an influencing factor at all in these injections, whether the prompt effect in these experiments is not due entirely to the direct and rapid manner in which the drug reaches the central nervous system. To test this point the drug was introduced, in a number of experiments upon normal frogs, through a cannula tied in the abdominal vein. The intact heart, receiving the drug almost immediately after its injection into the abdominal vein, sends it at once to the central nervous system, practically in the same direct and rapid manner as when it is injected through the bulbus arteriosus. These experiments, although not numerous (see table IV) show unmis-

TABLE IV

Injections through the abdominal vein into frogs with normal circulation. 5 per cent acid fuchsin, in frog saline solution used.

NUMBER OF EXPERIMENT	WEIGHT	DOSE PER GRAM FROG	TIME FROM OPERATION TO INJECTION	TIME FROM INJECTION TO FIRST CONVULSION	DURATION OF FLEXOR CONVULSION	DURATION OF EXTENSOR CONVULSION	TIME FROM INJECTION TO LOSS OF IRRITABILITY
	<i>gms.</i>	<i>mgm.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>
97	58	1/2	Never any convulsions (3 days).				112
98	55	1/2	Never any convulsions (2 days).				
100	65	3/4		11	-----	13-----	
101	67	3/4	No convulsions during 3 days.				
102	62	3/4	No convulsions during 3 days.				69
99	53	1		4	(Convulsions powerful and soon over)		
103	61	1	No convulsions during 3 days				300+
104	67	1		2	9		
105	63	1		43			323+
106	55	1		6	10		360+
107	55	1		11	11		348+

takably the striking difference which exists between the effects of the blood-free intra-aortic injections, and that of the intravenous injections. In two experiments with doses of $1/2$ *mgm.* per gram frog (of 5 per cent acid fuchsin in frog saline) the frogs showed no sign of a convulsion for over two days during which

time they were under observation. In three experiments, in which the frogs received intravenously $3/4$ mgm. per gram frog (of 5 per cent fuchsin in frog saline) two animals had no convulsions at all and one had moderately strong convulsions. Five frogs received intravenously 1 mgm. per gram frog (of 5 per cent fuchsin dissolved in frog saline). One of these frogs had no convulsions at all, and in another the convulsions set in about 43 minutes after the injection and ran a mild short course. In the remaining three frogs the convulsions set in rather early and ran a fairly strong course, which, however, could not be compared with the violence of the convulsions which invariably appeared after an intra-ortic injection of even such a small dose as $1/100$ mgm. per gram frog.³ It goes therefore without saying that the effects of intra-aortic injections of fuchsin are very much stronger than the effects of intravenous injections; we may even put down roughly that it is at least 100 times more effective.

Now it is evident that in injecting through the bulbus arteriosus, that is, into the common aorta, the solution is distributed through the body in the same manner and proportion and with about the same celerity as when it is injected into the heart through the abdominal vein. Accordingly, the main difference between the injection through the bulbus aortae and the intravenous injection consists in the fact, pointed out before, that in the intravenous mode of injection, blood enters the central nervous system at the same time with the fuchsin solution and, what is perhaps of greater importance, it continues to deluge the nervous system after the injection.

We may therefore say, that both series of experiments with intra-vascular injection sustain, in the first place, the claim that it is the blood of the cardiac circulation which reduces the toxicity of fuchsin, and that they, in a measure, support also the theory, that the blood contains substances which are capable of neutralizing to some extent the toxic action of acid fuchsin upon the central nervous system.

³ We ought to recall here the fact that when using 1 mgm. of the drug per gram frog, Barbour and Abel, saw occasionally even after injections into the lymph sac of normal frogs a fairly early onset of strong convulsions. (Compare, for instance, Experiment 2, p. 169, 1 c.)

IV. INTRAVENOUS INJECTION INTO ANIMALS DEPRIVED OF THE ANTERIOR PART OF THE BRAIN

In connection with the last mentioned experiments we shall record here briefly a few observations made with intravenous injections of fuchsin in frogs in which the anterior third of the brain was removed. In two frogs which received $1/100$ mgm. per gram frog and two others which received $1/50$ mgm. per gram frog no convulsions set in, for over twenty-four hours, that is, as long as they were observed. In four frogs which received intravenously $1/20$ mgm. per gram body-weight, two showed no symptoms and two had fairly strong convulsions, but in one of these the convulsions set in only after thirty-three minutes. Only one animal received $1/10$ mgm. per gram body-weight; it showed marked convulsions which began three minutes after the injection. See table v.

TABLE V

Injections through the abdominal vein into frogs with normal circulation but whose anterior one-third of the cerebrum had been removed. One per cent and $\frac{1}{10}$ per cent solutions of fuchsin in frog saline used.

NUMBER OF EXPERIMENT	WEIGHT	DOSE PER GRAM FROG	TIME FROM OPERATION TO INJECTION	TIME FROM INJECTION TO FIRST CONVULSION	DURATION OF FLEXOR CONVULSION	DURATION OF EXTENSOR CONVULSION	TIME FROM INJECTION TO LOSS OF IRRITABILITY
	<i>gms.</i>	<i>mgm.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>
81	60	$1/100$	Never any convulsions.				
82	77	$1/100$	Never any convulsions.				
83	67	$1/50$	No definite strong convulsions. A few mild indefinite convulsions.				
84	62	$1/50$	Never any convulsions.				
80	69	$1/20$	Never any convulsions.				
85	69	$1/20$	4	33	15	48	180
87	65	$1/20$	Never any convulsions.				
88	56	$1/20$		11	Fairly strong convulsions.		
86	65	$1/10$		3	19	115	152

While these experiments are only few in number, they show that even $1/20$ mgm. per gram body-weight, when administered intravenously, is capable of bringing out convulsions; while the smallest dose of the fuchsin, which, in the experiments of Barbour and Abel, occasionally brought out convulsions when

injected into the lymph sac, was not less than 0.35 mgm. per gram body-weight. That shows that the intravenous injection is much more effective, perhaps seven times more, than injections into the lymph sac. The rapidity of the onset of convulsions was, however, not markedly different between the two modes of injection. Again these experiments seem to show also a much greater increase in the toxicity of the fuchsin after elimination of the circulation than after the removal of the anterior part of the cerebrum. In the cardiectomized frogs even by injections into the lymph sac, 1/20 mgm. per gram body-weight was an infallibly convulsant dose, while this dose was not reliable (only two out of four) in decerebrated frogs even when the drug was administered intravenously. Remembering, in addition, that the intravenous injections are rather comparable with intra-aortic experiments, in which even such a small dose as 1/125 mgm. per gram body-weight proved to be an infallibly convulsant dose, it seems to be strikingly evident *that the absence of the circulation is a much stronger factor in favoring the production of convulsions by fuchsin than the removal of the anterior part of the cerebrum.*

V. DISCUSSION AND CONCLUSIONS

The experiments established in the first place the fact, that after removal of the cardiac circulation in frogs, an injection of fuchsin into the lymph sac, of even as small a dose as 1/20 mgm. per gram body-weight, brings out invariably violent convulsions in less than half an hour. In animals with normal circulation we know from the experiments of Barbour and Abel, that even 1 mgm. (per gram body-weight) of the fuchsin will very rarely bring on a convulsion in less than an hour. The experiments show, then, that (1) fuchsin is efficiently distributed through the peripheral, *lymph space mechanism*, and that, (2) when so distributed, it affects the central nervous system much more strongly than when it is brought there by the normal circulation. Other experiments have shown the additional fact, that if, after eliminating the heart, the fuchsin is injected into the common aorta, instead of into a lymph sac, even so small a dose as 1/125 mgm. (per gram body-weight) will invariably bring on violent convul-

sions. When the drug is injected intravenously in a frog with the cardiac circulation intact, even 1/2 mgm. (per gram body-weight) fails to bring on convulsions. The injections into the common aorta have this in common with the injections into a lymph sac of a cardiectomized frog, that the fresh blood is kept out from the central nervous system; but in the intra-aortic injections the drug is brought almost at once to the central nervous system while the transportation from the lymph sacs is necessarily slower. That, however, the rapidity of the transportation is not the chief cause of the great effectiveness of the intra-aortic injections, is shown by the fact, that for the intravenous injections, in which the rapidity of transportation to the central nervous system is practically as great as in intra-aortic injections, the effective dose must be nearly a hundred times larger. Apparently it is *the simultaneous and consecutive sending of the blood to the central nervous system, which makes the intravenous administration of fuchsin less toxic.*

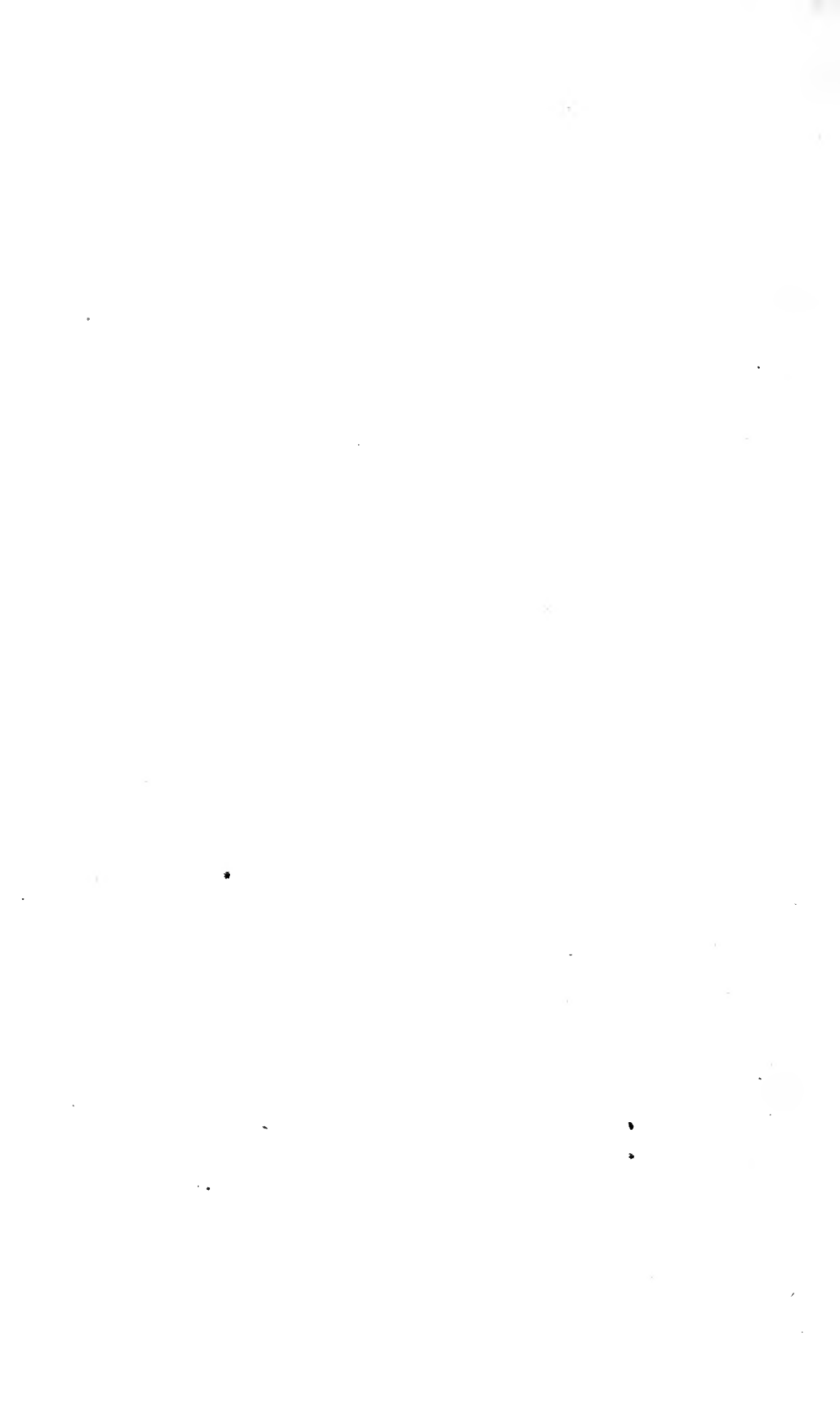
We know, then, two conditions which are capable of increasing greatly the toxicity of acid fuchsin for the frog: The removal of the anterior third of the brain (Barbour and Abel) and cardiectomy. From a comparison of the experiments we may safely say that the removal of the heart makes the drug much more toxic than the removal of the anterior part of the brain; for in injections into the lymph sac, 0.35 mgm. (per gram body-weight) is the (unsafe) minimum dose after removal of the brain, while 0.05 mgm. (per gram body-weight) is the (infallible) minimum dose after the removal of the heart. A similar striking difference between the minima of toxicity was found to exist between the intravenous injection in frogs with the anterior part of the brain removed and the intra-aortic injections in frogs with heart removed and brain intact.

As an explanation for the influence of the removal of the heart we offer the following theory. The blood receives from various organs of the body a variety of secretions some of which, we know, are capable of neutralizing various toxins and poisons. It is therefore possible that it is some such substance in the blood which keeps down the toxicity of acid fuchsin in the normal animal.

After elimination of the circulation and, with it, the neutralizing activity of the hypothetical substance, the acid fuchsin gets a chance to develop its toxicity. The increasing effect of the removal of the anterior part of the brain, Barbour and Abel explain by the assumption, that this part of the brain, when present, exerts an inhibitory effect upon the excitability of the cord; the removal of this part removes, therefore, or decreases the restraint of the inhibition; hence the greater convulsant effect of the drug.

May we not explain the convulsant action of both procedures by a single hypothesis? For instance, may we not assume, that the removal of the heart causes a complete anemia of the anterior part of the brain, and thus eliminates the inhibitory activity of this part as efficiently as if it would have been cut off? We believe, that this hypothesis is not acceptable for the following reasons. In the first place, it is not a very plausible assumption that the anemia will reduce the (inhibitory) irritability of just that part alone. But granting even the admissibility of such a hypothesis, the anemia certainly could not destroy the part more thoroughly, than it is done by its entire removal. How, then, could the removal of the heart cause a definitely greater toxicity of the drug than the removal of the brain, if the latter procedure be the only cause for the rise in toxicity? But to meet this objection, we may, perhaps, assume further, that the anemia removes inhibitory mechanisms also in other parts of the brain, besides that of its anterior part, and hence the greater effect of the removal of the heart. But if this were true, the simple removal of the heart alone ought to lead, surely, to a definite manifest hyperexcitability—which is absolutely not the case. Frogs after removal of their hearts, pass from a normal condition, through a stage of increasing depression, to absolute paralysis, without showing at any time, an increase in the reflex irritability.

We shall not try to invent more unifying hypotheses and controvert them. For the present we are willing to accept both theories as temporary interpretations of the undeniable facts. But we shall refrain from dwelling here theoretically upon the particulars of the mutual relations of both theories; future experimental work will undoubtedly render better services than mere additional hypotheses.



THE PHYSIOLOGICAL ACTION OF CYTISINE, THE ACTIVE ALKALOID OF LABURNUM (CYTISUS-LABURNUM)

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I. HISTORICAL AND INTRODUCTORY

Cytisine is an alkaloid, present in a number of plants, which was first isolated from the seeds and other parts of the common Laburnum tree (*Cytisus laburnum*): its presence in the latter has led to numerous cases of accidental poisoning.¹

Most of the cases occur in children, who eat the seeds in play. Radziwillowicz¹ in 1888 collected accounts of 131 cases, including five fatal ones. The most recent detailed account of the symptoms which we have seen was given by Vallette,¹ who in 1908 attended three women who had eaten a dish in which laburnum flowers had been used as a flavoring agent, in mistake for those of *Robinia pseudacacia*. The most constant symptom appears to be vomiting, succeeded by prostration and torpor, which may or may not be preceded by a stage of excitement. Other symptoms described are delirium, hallucinations, mydriasis, muscular twitchings, convulsions, salivation, diarrhoea, vertigo, pallor and cold sweats. In Vallette's patients the first symptom was a feeling of numbness in the hands. Death, when it occurs, is due to respiratory paralysis.

¹ Cf. Radziwillowicz: *Arb. d. pharm. Inst. z., Dorpat II*, p. 56, 1888. Vallette: *Rev. med. de la Suisse Romande*,—1908, p. 366. Also various authors in *The Lancet*, 1877, ii, pp. 341 and 414; 1901, ii, p. 491; 1905, ii, p. 635; and the *Brit. Med. Journ.*, 1870, i, p. 79; 1882, i, p. 199, 1883, i, p. 1117.

The alkaloid was first prepared and named by Gray,² isolated pure by Husemann and Marmé,³ and further studied by others, including Partheil,⁴ who assigned to it the accepted formula $C_{11}H_{14}N_2O$, and more recently, Freund and his pupils.⁵

Its action on animals has been described by Gray, Husemann and Marmé,⁶ Cornevin,⁷ Prevost and Binet,⁸ and Radziwillowicz.¹ Bradford⁹ also described the action of "ulexine," an alkaloid obtained by Gerrard from the seeds of the common gorse, and since shown to be identical with Cytisine. Most of the accounts agree as to the obvious symptoms of poisoning by Cytisine. These are described as a stimulation of medullary centres resulting in dyspnoea, salivation and vomiting, and a very large rise of blood-pressure, muscular twitchings and tremors, partly central, partly peripheral in origin, succeeded by weakness, lethargy and narcosis. With intravenous injection of a few milligrammes the stage of excitation is said to be succeeded by paralysis of the centres, death being due to paralysis of the respiratory centre, so that life can be prolonged indefinitely by the application of artificial respiration. Bradford obtained the rise of blood-pressure in the dog after section of the cervical cord. Most observers describe a curare-like action, which, according to Radziwillowicz, is produced with relative ease in the cat and dog, as compared with the frog. According to Bradford the muscular tremors are at least partly of peripheral origin, as they continue after response to motor nerves is abolished, and persist for some time in a completely severed limb. Increased diuresis is mentioned by Bradford and by Radziwillowicz. Rodents are relatively very insensitive to the poison (Prevost and Binet, Radziwillowicz). On some points there is lack of agreement between different observ-

² Edinburgh Med. Journ., vii, ii, pp. 908, 1025, 1862.

³ Zeitsch. f. Chem., i, p. 161, 1865.

⁴ Berichte d. deutsch. Chem. Gesell., xxiii, p. 3201, 1890.

⁵ Ibid., xxxiv, p. 615, 1901; xxxvii, p. 16, 1904; xxxix, p. 814, 1906.

⁶ Loc. cit. Also Marmé: Nachr. d. kön. Gesell. z. Wissensch., z. Göttingen, 1887 (Ref. Therap. Monatsh., 1887, p. 156).

⁷ Comptes Rendus, 1883, p. 777.

⁸ Rev. med. de la Suisse Romande, 1887, pp. 516 and 553, and 1888, p. 670.

⁹ Journ. of Physiol., viii, p. 79, 1887.

ers. Thus Prevost and Binet noted the absence of effect on intestinal peristalsis, though a stimulant effect on this is described by Radziwillowicz. Prevost and Binet, again, describe the motor nerves in the frog as being paralysed before the vagus effect on the heart. The reverse relation was, apparently, observed by Radziwillowicz, who emphasises the fact that the curare-action is not obtained in the frog with small doses, though larger doses produce it. His description of the comparatively early paralysis of the fore-limbs in the frog, which show a cataleptic¹⁰ condition while the hind-limbs are still capable of feeble movement, is very suggestive. The same observer found that 10 mgms. of Cytisine nitrate, given subcutaneously, caused stiff extension of the hind-limbs in a fowl; 12 mgms. killed it. He also observed powerful, peristaltic contraction of the uterus of a pregnant cat as the result of injecting 1 mgm. intravenously. Both Marmé and Radziwillowicz found that the alkaloid was excreted unchanged in the urine.

It is evident that the description given by these observers of the action of Cytisine might be transferred to that of nicotine¹¹ without any essential alteration. The general comparison was not made, however, though Prevost and Binet remark that, on direct application of Cytisine to the frog's heart, a momentary arrest is produced "similar to that produced by nicotine." Its pharmacological affinities were regarded as being rather with curare and, for no very clear reason, strychnine; Radziwillowicz concluding that its action is intermediate between those of strychnine and curare, but nearer that of the former.

Our own experiments confirm the impression made by perusal of the accounts of previous workers that the action of Cytisine is closely similar to that of nicotine. It may be recalled that a

¹⁰ The statement is that the fore-limbs "wie gelähmt verharren bei jeder Lageveränderung in derselben Stellung." The meaning is not clear, but we imagine that a description is intended of the cataleptic condition, which is very easily observed.

¹¹ We have not attempted to give the individual authority for the different details of the action of nicotine to which reference is made. Most of the points to which we refer will be found in the paper by Langley and Dickinson, *Journ. of Physiol.*, xi, p. 265, 1890, in which full references to earlier literature are given.

similarly close or even closer resemblance to nicotine in action was observed by Edmunds¹² in the case of Lobeline.

II. EXPERIMENTAL

For our supply of Cytisine we are indebted to our colleague Mr. Ewins, who extracted it from Laburnum seeds. For our experiments the alkaloid was dissolved in water, in which it is readily soluble, the strongly alkaline solution being then exactly neutralised with HCl and diluted to contain 1 per cent of the base. Further dilutions were made with physiological saline from this stock solution. Our observations on the general toxic effects of Cytisine correspond in the main with those of previous observers. Points of difference can be noted in dealing with the separate systems.

Skeletal muscles. Cytisine, like nicotine, causes muscular tremors in mammals when injected intravenously. These are doubtless partly central in origin, being depressed by chloroform or other anaesthetic and by pithing the cord. The characteristic twitching of the cat's ears, which is one of the first visible effects of an intravenous injection of nicotine, lobeline or hordenine-methiodide in that animal, is not produced by Cytisine. After doses varying from 6 to 10 mgms. in different experiments we found stimulation of the sciatic nerve in the anaesthetised cat quite ineffective: the muscles still responded well to direct faradisation. In curare-like action on the cat, therefore, Cytisine is about as powerful as nicotine. The rabbit, as noted by previous observers, is relatively resistant. Five milligrammes intravenously, in a rabbit of $2\frac{1}{2}$ kilos, produced a mere trace of muscular twitching. Ten milligrammes subsequently caused violent general twitching, passing into fatal convulsions. The jaw muscles continued to twitch long after the death of the animal.

We have noted that a stiff extension of the legs, recalling that produced by nicotine, was observed by Radziwillowicz in the fowl as the effect of injecting Cytisine. In a fowl anaesthetised with ether, with the gastrocnemius tendon of one leg isolated and

¹² American Journ. of Physiol., XI, p. 79, 1904.

attached to a lever as described by Langley,¹³ we observed a marked and persistent tonic contraction of the muscle when 2 mgms. of Cytisine were injected intravenously. Our impression is that the effect was less than that which a corresponding injection of nicotine would have produced. It is impossible, however, to be certain of this, since the effect of either alkaloid is reduced or abolished by a previous injection of the other, so that the two cannot be compared on the same animal. The effect of Cytisine on the frog is strongly reminiscent of that of nicotine. One-half milligramme injected into the dorsal lymph-sac caused slowness of movement in four minutes. In five minutes the fore-limbs were paralysed, being trailed alongside the body when the animal attempted to jump, so that the nose was thrust against the table. In six minutes a pronounced cataleptic condition of the fore-limbs was present: respiration had also ceased. Slight power of voluntary movement of the hind-limbs was retained long after this, however, and they still exhibited weak twitches thirty minutes after the injection. The frog was then pithed and dissected, when it was found that the muscles of the hind-limbs responded well to stimulation of the sciatic plexus. In another frog 2 mgms. produced a complete curare-like effect, the muscles being, however, still fully responsive to direct excitation. The action on isolated muscles of the frog was compared with that of nicotine. Pairs of sartorii were isolated from several frogs and placed in tap-water saline (0.6 per cent). They were fixed in turn in a Keith-Lucas¹⁴ muscle-trough filled with saline, and records taken of the contraction produced when the saline was replaced by 0.1 per cent solution of nicotine or Cytisine made up with saline. In each case the action of nicotine on one sartorius was compared with that of Cytisine on the other muscle from the same frog. One-tenth per cent Cytisine always produced a well-marked tonic contraction, but this was in every instance slower in onset, lower in maximum and more evanescent than the effect of nicotine on the corresponding muscle. Nicotine, added subsequently to

¹³ Journ. of Physiol., xxxiii, p. 380, 1905.

¹⁴ Journ. of Physiol., xl, p. lxiv (Proc. Phys. Soc.), 1910.

subsidence of the Cytisine contraction, or Cytisine after nicotine, produced no effect. Similar results were obtained with the gastrocnemius and the flexor longus digitorum.

The excitatory action of Cytisine on skeletal muscle is, on the whole, therefore, similar to but weaker than that of nicotine: in curare-like action the two appear to be nearly identical.

Respiration. Previous observers have described a stimulant followed by a paralytic action on the respiratory centre. This is easily observed in the anaesthetised cat, in which animal the intravenous injection of 1 to 2 mgms. causes violent respiratory movements followed by cessation of respiration. If artificial respiration be applied the normal respiration begins again in a few minutes. In a rabbit, on the other hand, in which we made the injection by the ear-vein without anaesthesia, we observed no primary stimulation of the respiratory centre. Immediately after the injection the respiration ceased: with the smaller doses (up to 5 mgms.) it was soon gradually resumed; after an additional dose of 10 mgms. it was permanently abolished, the convulsions ensuing being possibly due in part to asphyxial stimulation of the cord.

Heart and circulation. The rise of blood-pressure recorded by all previous observers,¹⁵ has by them been generally attributed to stimulation of the medullary vaso-motor centre. Bradford pointed out that a rise of blood-pressure was still produced by ulexine after section of the cervical cord. We find that after extirpation of the whole cord of a cat by pithing, the pressor effect is obtained practically unimpaired. The first effect on the circulatory mechanism of injecting 0.25 to 1 mgm. of Cytisine intravenously, even with the vagi cut, is inhibition of the heart: this may pass off rapidly as the blood-pressure begins to rise, or, especially with larger doses, may persist for a large part of the ascending limb of the pressure-curve. Sooner or later it gives way to pronounced acceleration. The combined cardio-acceleration and vaso-constriction produced by 0.25 mgm. drive the

¹⁵ The effect was denied by Prevost and Binet in their first and main paper, but they subsequently observed it.

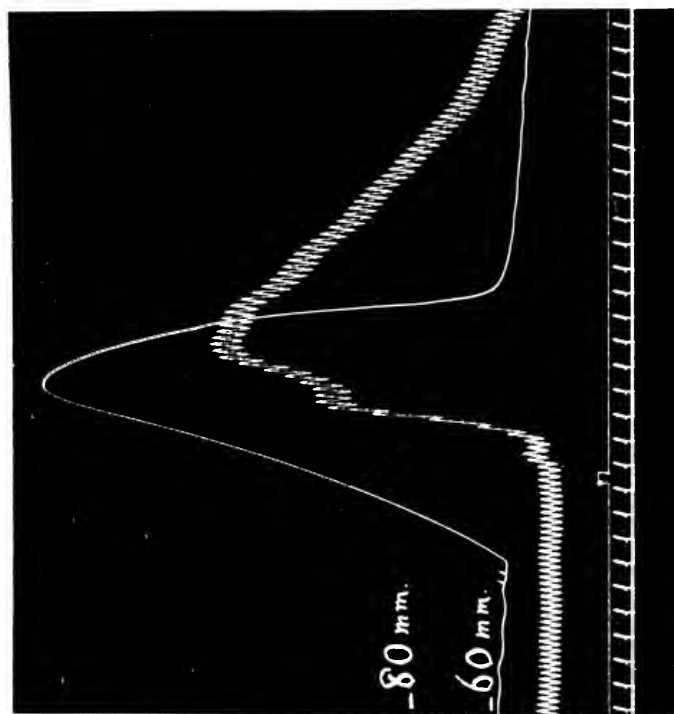


FIG. 1. Cat: brain and cord pitheal. Bladder volume-record and carotid blood pressure. Effect of 0.25 mgm. nicotine intravenously. (0.25 mgm. nicotine and 0.25 mgm. Cytisine injected previously.)

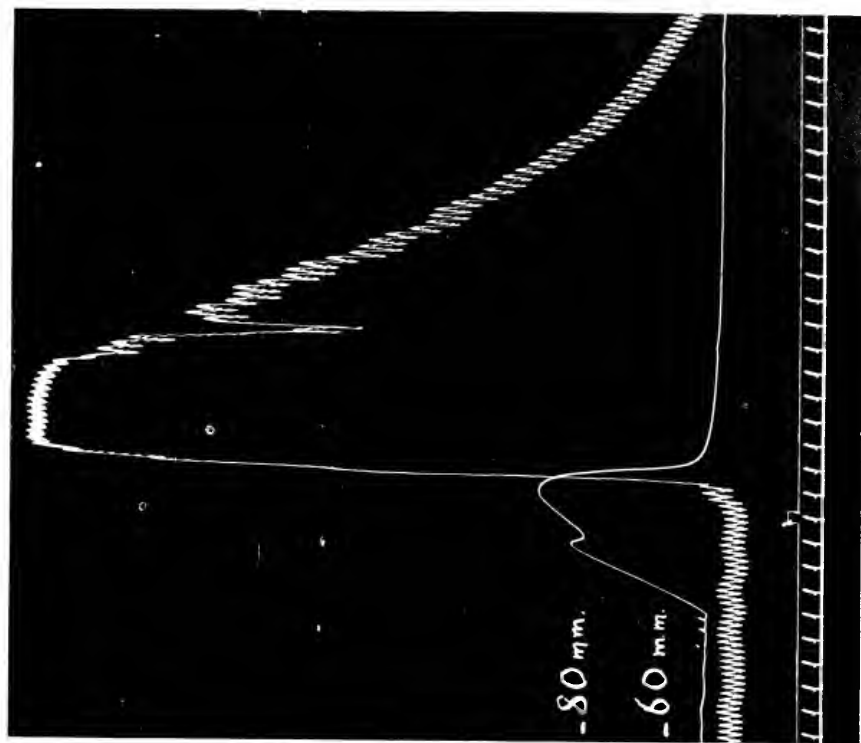


FIG. 2. Continuation of experiment shown in fig. 1. Effect of 0.25 mgm. Cytisine intravenously. Note that the effect on the blood pressure is much greater, that on the bladder much less than in fig. 1.

pressure up to the maximum possible to the cat under the conditions of the experiment. The effect is evanescent, and reproduced in diminishing degrees by successive doses. After 20 to 30 mgms. in all have been given further injections produce no effect on the blood-pressure.

After sufficient nicotine to render a cat irresponsive to further nicotine injection, Cytisine is without effect on the blood-pressure; an animal similarly paralysed by Cytisine is unaffected by nicotine. Each, therefore, in sufficient quantity paralyses the structures which the other stimulates. After a sufficient dose of ergotoxine (about 4 mgms. is usually needed in a cat) Cytisine produces a fall in place of a rise of blood-pressure.

For the comparison of the pressor activities of the two alkaloids small doses must be employed. In a cat with completely pithed central nervous system 0.25 mgm. of Cytisine is found always to produce a more rapid and greater rise of pressure than that produced by the same dose of nicotine, in whichever order they are given. As a first injection of this quantity of Cytisine frequently produces a supra-maximal effect, the difference becomes more marked with later injections, the effect of nicotine thus appearing to be more rapidly paralysed (see figs. 1 and 2). The effect of a given submaximal dose of Cytisine can, however, still be surpassed in height by that of a sufficiently large dose of nicotine, and as soon as sufficient nicotine, or Cytisine, has been given to annul the effect of further injections of nicotine on the blood-pressure altogether, no amount of Cytisine will produce any further rise. In primary stimulant action, then, on peripheral sympathetic neurones concerned with cardio-acceleration and vaso-constriction, Cytisine is considerably more active than nicotine: in secondary paralytic action on the same structures the two are apparently about equal. We hope to study the relation between the two actions in greater detail: there are certain points arising in the comparison of which the meaning is not yet clear. The initial stimulation of the vagus inhibitor mechanism is succeeded, when larger doses (e.g., 10 mgms.) are given, by paralysis of the effect of excitation of the vagus trunk. In this respect again, the action is like that of nicotine;

the likeness is rendered more striking by the production after 2 mgms. of Cytisine, and especially in a cat under paraldehyde, of the phenomenon of reversed vagus action on the heart (fig. 3) which we described in a recent paper,¹⁶ and which was likewise produced by about 2 mgms. of nicotine.

Application of a few drops of 1 per cent Cytisine to the frog's heart causes transitory inhibition followed by return to the normal or slightly accelerated rate. Stimulation of the vagus then produces only acceleration or augmentation of the heart-beat.

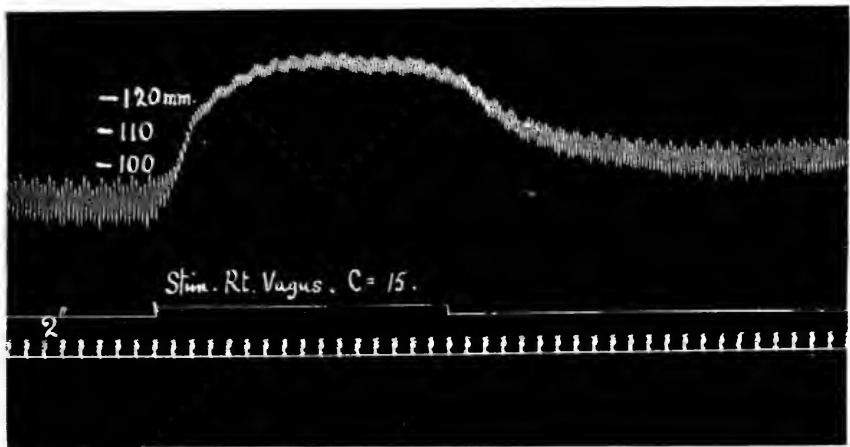


FIG. 3. Cat: Paraldehyde. Carotid blood-pressure. Reversed effect of the vagus on the rate of the heart-beat after 2 mgms. Cytisine.

Alimentary canal. Vomiting is one of the earliest and most characteristic symptoms of the action of Cytisine on the dog or cat. It is incompletely suppressed by anaesthesia, vomiting efforts of some vigour being produced in a cat under ether by the injection of 2 mgms. intravenously. Sometimes they are effective to the extent of producing regurgitation of part of the more fluid contents of the stomach.

The small intestine of the cat exhibits inhibition during the rise of pressure produced by Cytisine, followed by some exagger-

¹⁶ Journ. of Physiol., XLI, p. 1, 1910.

ation of the normal pendulum movement as the pressure returns to the normal (fig. 4). The effect, like that on the blood-pressure, becomes progressively weaker with repeated injections. The whole effect is qualitatively indistinguishable from that produced by nicotine under the same conditions: a quantitative comparison

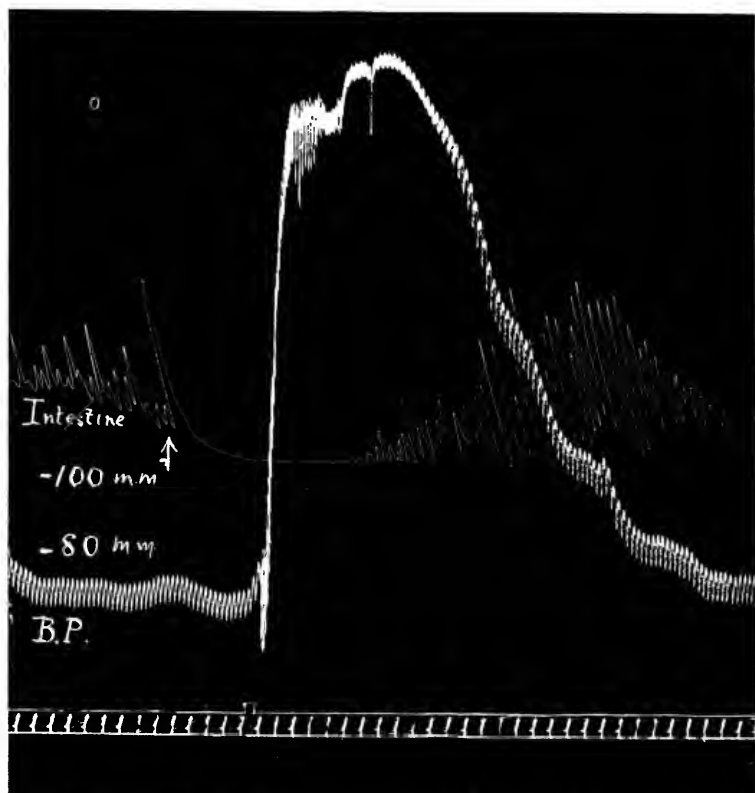


FIG. 4. Cat: Brain pithed. Balloon record from jejunum: carotid blood-pressure. Effect of 2 mgms. Cytisine intravenously. (Note the initial inhibitor effect on the rate of the heart-beat.)

was not made in this instance. In the rabbit, the effect of Cytisine on the bowel, again like that of nicotine, is predominantly motor. Intravenous injection of 2 mgm. into the jugular vein of a small rabbit, with pithed brain, caused a pronounced writhing

peristalsis of the whole of the small and large intestines, including the caecum.

By recording the longitudinal contractions of an isolated loop of rabbit's jejunum we observed, however, a distinct quantitative difference in the action of the two alkaloids. Two milligrammes of Cytisine added to the bath of 250 cc. Ringer's solution, caused momentary inhibition of pendulum movement, followed by increase of tonus and rhythm, which after about 30 seconds was succeeded by weak inhibition of both (fig. 5). On changing the Ringer's solution, the tonus and rhythm were slowly recovered. Two milligrammes of nicotine were then added, and caused, after a slight transient inhibition, a very much larger and more persistent tonic-contraction, also succeeded by an inhibitor phase (fig. 6). After recovery from this, in clean Ringer's solution, a further dose of 5 mgms. of Cytisine caused but weak motor and inhibitor effects, much weaker than those produced by a subsequent 2 mgms. of nicotine. Cytisine, therefore, is much weaker than nicotine in motor action on the rabbit's intestine.

We shall see that the same is true of the motor effects of the two alkaloids on the cat's bladder.

Salivary glands. Salivation has been mentioned by several observers as a prominent symptom of poisoning by Cytisine in the cat and dog. The fact that vomiting is also one of its most constant effects suggests the probability that the salivation may be largely reflex, or at any rate central in origin. It is not, however, wholly so, since Cytisine again resembles nicotine in producing some flow of saliva in the anaesthetised dog or cat after section of the chorda tympani. Unless the first dose is very small, subsequent injections produce little or no flow.

The effect of the first small dose of nicotine or Cytisine depresses to such an extent the effect of subsequent similarly small doses of either alkaloid on the salivary flow, that we found it impossible to establish a comparison between their activities in this direction.

We found the effects of chorda stimulation in the dog only slightly reduced after intravenous injections amounting to 3 mgms. After 18 mgms. in all the immediate effect of stimulation was very

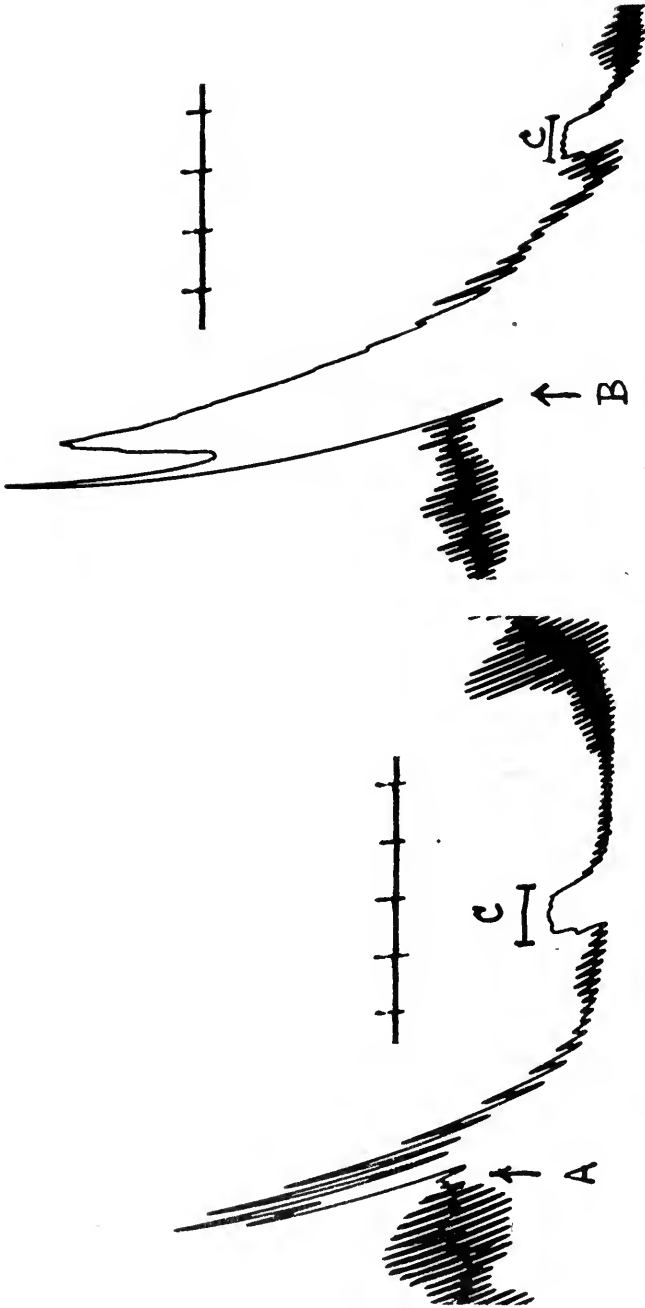


Fig. 5. Isolated loop of rabbit's small intestine, suspended in 250 cc warm oxygenated Ringer's solution, recording longitudinal contractions. At A added 2 mgms. Cytisine to the bath. At C fresh Ringer.

Fig. 6. Continuation of record shown in fig. 5. At B added 2 mgms. nicotine to bath. At C fresh Ringer.

slight. The ganglion-cells, however, were not completely paralysed; for not only was a slight secretion produced during the stimulation, but after the stimulation was stopped, secretion continued for some time and at a considerably greater rate. Renewal of chorda stimulation during this after-period promptly reduced the rate, which again became accelerated as soon as the stimulation was stopped. The reversed action when once obtained could apparently be repeated indefinitely, though the effect of the Cytisine would doubtless have passed off in time if the experiment had been continued long enough. If a prolonged stimulation were given the flow became gradually accelerated during its progress, but there was a further and more sudden acceleration when the stimulation ceased.

A similar reversal of chorda action was observed in the cat with smaller doses of Cytisine.

The details of some of these experiments have been published elsewhere.¹⁸ The fact that the secreto-motor effect of the chorda may be mainly an after effect, after incompletely paralytic doses of nicotine, was shown by Langley in 1890.¹⁷ The inhibition of the delayed secretion by restimulation is clearly an analogous phenomenon to the reversed vagus effect on the cat's heart, which we described as occurring after nicotine, tropine, curare, hordenine-methiodide,¹⁵ and now after Cytisine, and the similarly reversed effect of the pelvic nerve on the cat's bladder after curare described by Langley.¹⁹ The immediate interest of the matter for us is the addition of another point of similarity between the action of Cytisine and that of nicotine.

The eye. In the dog and cat the primary effect on the eye, of an injection of Cytisine, like that of nicotine, is similar to that of stimulating the cervical sympathetic—dilatation of the pupil, retraction of the nictitating membrane and widening of the palpebral fissure. The retraction of the nictitating membrane is brief, and is soon succeeded by a movement forward, so that the membrane, after several milligrammes of the alkaloid, covers

¹⁷ Journ. of Phys., XI, p. 123, 1890.

¹⁸ Journ. of Phys., XLIII, p. 196, 1911.

¹⁹ Journ. of Phys., XL, proc. Phys. Soc., p. lxii, 1910. Also XLIII, p. 125, 1911.

one-half to two-thirds of the visible portion of the eyeball. With doses up to 10 mgms. in the cat the pupils remain large. In the rabbit, on which we only made one experiment, and that on the intact unanesthetised animal, 2 to 5 mgms. by the ear-vein caused constriction of the pupil and retraction of the nictitating membrane. In a dog, which received 30 mgms. hypodermically, the pupils dilated and the nictitating membranes gradually became greatly prolapsed, so that the eyes must be rotated upwards and outwards to permit vision. In all these respects the action is again closely parallel to that of nicotine. So also is the paralytic effect on the cells of the superior cervical ganglion. Application of 1 per cent Cytisine to the superior cervical ganglion of a cat under ether caused a brief effect of sympathetic stimulation on the eye followed by complete paralysis of the cervical sympathetic nerve to electrical stimulation up to the ganglion. Stimulation of the branches from the ganglion to the internal carotid produced the normal effect. The same effect, as the result of intravenous injections, is shown in the following record, which illustrates incidentally other features of the action of the alkaloid.

Cat.

- 11.10 a.m. Chloroform. Then A. C. E. throughout. Tracheotomy. Cannula in femoral vein, by which all injections were made.
- 11.30 a.m. Pupils medium: nictitating membrane partly forward. 1 mgm. Cytisine. Widening of pupil and retraction of memb. nict. The latter then moved forward with continued widening of the pupil. A few deep respirations: then normal again.
- 11.35 a.m. Pupils nearly maximal: memb. nict. three-quarters prolapsed.
- 11.37 a.m. Two milligrams. A very few deep respirations. Then vomiting movements during which respiration ceases. After a short period of artificial respiration it is resumed normally. Heart-beat very slow. Right cervical sympathetic cut and isolated for stimulation.

- 11.52 a.m. Cat having artificial respiration with A. C. E. Pupil practically maximal. Stimulate cervical sympathetic with coil at 20 cm. Good retraction of nictitating membrane.
- 11.54 a.m. Five milligrams Cytisine. Memb. nict. retracts slightly and then returns. Pupils maximal. Slight tremors of paws.
- 11.55 a.m. Stimulate cervical sympathetic with coil at 20 and 15 cm. No effect. With coil at 10 cm. trace of retraction of memb. nict.
- 11.57 a.m. Stimulate sciatic (coil at 10 cm.)—feeble twitches of foot.
- 11.58 a.m. Stimulate cervical sympathetic (coil at 15 cm.)—memb. nict. retracts very slightly and then returns during stimulation, retracting again on cessation of stimulus. Repeated with identical effect.
- 12.2 p.m. Five milligrams Cytisine.
- 12.4 p.m. Stimulate sciatic, coil at 10 cm. No effect. Same stimulus directly to thigh muscles—normal contraction.
- 12.6 p.m. Stimulate cervical sympathetic, coil at 10 cm.—very weak and slow retraction of memb. nict. Repeat—no effect. Isolate branches from ganglion to internal carotid and stimulate with coil at 20 cm.—normal retraction of nictitating membrane.

Cytisine, therefore, like nicotine, produces its stimulant effects when directly applied to the ganglion-cells which it ultimately paralyses. On the other hand we find that its dilator action on the pupil, at any rate, is, again like that of nicotine, not wholly due to action on the superior cervical ganglion, since the pupil still dilates when Cytisine is injected intravenously after the ganglion has been removed.

The uterus. We have experimented with Cytisine on the uterus of the cat only. In this animal, as might be expected, the action of Cytisine changes like that of the sympathetic nerve supply and of adrenaline or nicotine, being inhibitor in the virgin organ, motor in the pregnant, when the uterus is in its natural relations and the alkaloid administered intravenously. Like nicotine, Cytisine in small doses has practically no effect on the cat's uterus isolated from the body.

The urinary bladder. We have examined the effect on the cat's bladder. In all our experiments on this organ the central nervous system of the cat was destroyed completely by pithing. This excluded indirect effects from stimulation of centres in the cord; but the bladder under these conditions having but little initial tone, the effect obtained was only that on the motor ganglia connected with the pelvic nerves. Probably, as in the case of nicotine, a secondary inhibition from the sympathetic ganglia could be demonstrated under more favorable conditions of initial tonus. Our main object was to compare the extent of the main motor effect produced by Cytisine with that produced by an equal dose of nicotine. The comparison reveals a curious contrast. When small alternating doses of the two alkaloids are given at regular intervals, the contractions of the bladder produced by Cytisine are found to be regularly much less than those produced by nicotine, whereas Cytisine, as mentioned above, regularly produces a quicker and greater rise of blood-pressure (see figs. 1 and 2).

III. SUMMARY AND DISCUSSION

The results of this investigation may be roughly summarised in the statement that, in nearly every respect, the action of Cytisine is qualitatively indistinguishable from that of nicotine. Such small points of difference as its failure to produce the characteristic twitching of the ears in the cat have possibly a diagnostic, but at present no great theoretical importance. We have indicated certain quantitative differences in the action of the two alkaloids, such as the more powerful pressor action of Cytisine in the cat, and its less powerful action on the rabbit's intestine and the cat's bladder. In one case in which we compared the two, nicotine produced a more marked primary inhibition (vagus) effect on the cat's heart than Cytisine. The fact that both alkaloids paralyse ganglia in succession to their stimulation makes it useless to attempt to give numerical expression to their relative stimulant activities on any particular organ. We have contented ourselves, therefore, with this mere indication of the order of

their activities in these few instances. Still more difficult would be a comparison of their potency in producing secondary paralysis of ganglia, since, for obvious reasons, the two cannot be compared in this respect on the same animal. As far as individual differences permit us to judge, we should say that their activities in this direction on the cat and dog are of the same order. The affinity of the action of Cytisine with that of nicotine is further shown in the production of a reversed action of certain autonomic nerves under the influence of incompletely paralytic doses, such as has previously been demonstrated with nicotine, curare and other alkaloids of the same group.

One of us has previously shown the resemblance of the action of hordenine-methiodide²⁰ to that of nicotine, with which it has no obvious points of chemical similarity. The similarity with nicotine in action is very much closer, however, in the case of Cytisine: so close, indeed, that we doubt whether any instance of such exact parallelism is known to exist in the case of substances which are not close chemical relatives, except in the case of the apparently still closer resemblance in action to nicotine exhibited by Lobeline. At present but little is known of the constitution of Cytisine, though there are indications that its structure in some points resembles that of nicotine. An exact knowledge of its structure may give interest to the small points of difference between the two actions as well as to their general similarity.

On the basis of less complete pharmacological investigations certain therapeutic uses have been suggested and tried for Cytisine. Thus Radziwillowicz tried it in cases of migraine with low blood-pressure, and Bradford tried ulexine as a diuretic. Our experiments lend no support to the suggestion of its being therapeutically valuable. For physiological investigations it could be used in place of nicotine, and would have certain advantages in its easy preparation, with large yield, from the readily and cheaply obtainable *Laburnum* seeds; in the ease with which it can be purified—the alkaloid itself crystallises well and can be distilled under reduced pressure; and in its comparatively great stability.

²⁰ Barger and Dale: *Journ of Phys.*, xli, p. 35, 1910.

THE INFLUENCE OF SODIUM IODOXYBENZOATE AND SODIUM CYANIDE UPON AN ALLERGIC REACTION OF INFLAMMATORY CHARACTER

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It is conceivable that allergic (1) phenomena might be influenced in various ways. For instance, the biological reactions leading to a production of toxins might take their course while one or more of the effects of these toxins may be counteracted or the toxins may be neutralized or destroyed. Furthermore, the course of the biological reactions, as for instance, the union of allergen with ergin, resulting in the production of toxins, might be influenced, inhibiting or increasing the production of these toxins. Other influences might be directed toward the removal of ergins already formed, or the production of ergins might be inhibited or furthered.

The question arises whether it is possible to influence allergic reactions by means of chemical substances of known composition foreign to the organism. Aside from the self-evident, ultimate, clinical value of such studies, they may serve to elucidate different problems of allergy. For instance, as the study of specific accelerators and inhibitors has been of great value in the study of ferments, so the influence of drugs on allergic reactions may aid in the solution of such problems as the relationship of localized to general allergic reactions.

Of previous investigations concerning the influence of chemical substances on allergic phenomena we may cite the effect of atropin on the anaphylactic shock in guinea-pigs (2). Here the drug apparently does not interfere with the reactions resulting

in the production of toxins, but it counteracts an effect of the toxin, preventing the bronchial spasms. Similarly it is not unlikely that the effect of barium chloride (24) preventing and counteracting the anaphylactic fall of blood pressure in dogs is not attributable to an influence on the mechanism of the allergic reaction. The mode of action of chloralhydrate (3) in the prevention of anaphylactic shock in guinea-pigs is less clear. The fact that guinea-pigs protected against the shock by this drug do not become antianaphylactic indicates that it influences the mechanism of the allergic reactions. Again, another mode of action is signified in the experiments of Hektoen (4) where sodium-iodoxybenzoate increased the production of haemolytic antibodies in the dog. Here belong the investigations of Agazzi (5) with regard to the stimulation of the production of agglutinins by arsenic preparations and those of Madsen and Tallquist (6) concerning the influence of pyrocin and pyrogallol on the production of lytic antibodies. Friedberger and Hartoch (7) prevented the anaphylactic symptoms in actively and passively sensitized animals by previous administration of a concentrated sodium chloride solution. Friedberger in conjunction with Vallardi, Nathan, (8), and others has shown that the anaphylactic symptoms are due to the formation of an anaphylatoxin, for the production of which complement is necessary. The influence of the sodium chloride is attributed to an interference with the action of the complement.

In our investigations we used mainly the intracutaneous reaction of rabbits sensitized to horse serum, in a like manner as Knox, Moss and Brown (9). This reaction is known as the phenomenon of Arthus (10) although Arthus worked with subcutaneous, not intracutaneous, injections.

A rabbit sensitized to horse serum responds to an injection of a small amount of horse serum into its epidermis with a local reaction at the place of injection. This reaction consists of a swelling mostly accompanied sooner or later with reddening and heat. Frequently more or less pronounced haemorrhages occur and occasionally the center of the reaction becomes necrotic, even when using diluted serum.

It was found desirable to find some convenient expression for the intensity of the reaction. For this purpose we measured the area of the swelling, marking its borders as accurately as possible by the aid of palpation and measuring it in two diameters, taking the average of these two measurements as the diameter of the reaction. The measurements are given in millimeters. Frequently the measurements can be made very accurately, the swelling being very sharply defined. But this is by no means always the case and the measurements become the less accurate the more gradual the transition of the swelling to normal skin. We wish to state that our measurements do not lay claim to mathematical accuracy. The decimals of our tables are the result of making averages, the actual measurements were not made to fractions of a millimeter. As a rule the size of a reaction corresponds well with the degree of infiltration. But here again exceptions are not too infrequent. So it may occur that a reaction may cover a rather extensive area while the swelling is much less pronounced than that of another reaction decidedly smaller in size, leaving no doubt that the reaction covering a smaller area is really more intense. Furthermore, the infiltration may not be uniform and its shape irregular. All the factors mentioned have to be taken into consideration before arriving at a decision with regard to the intensity of a reaction. Nevertheless, on the whole the measurements of the infiltrated areas give a fair representation of the intensity of the reactions, particularly the averages taken from several animals.

It was necessary to gain some information with regard to the variations of the intensity of the reactions at repeated injections in the same animal. The rabbits were sensitized by giving two intravenous injections of 1.0 cc. horse serum followed by repeated intracutaneous injections of 0.01 to 0.04 cc. This method of sensitization had proved successful with regard to the intracutaneous reaction in experiments of Moss and Brown (11). These intracutaneous injections were given in different places of the shaved abdomen, keeping in mind the result of von Pirquet (12), who showed that in the case of the cutaneous tuberculin reaction there exists a difference between reactions obtained in places not

used hitherto and those where the tuberculin had been applied previously. All of the measurements were obtained from reactions on the abdomen. A number of experiments were made, giving the injections in the skin of the shaved ear. If the reactions do not exceed a certain limit, they can be traced here very prettily and accurately, particularly in transparent light. But the reactions in the ear may readily become too extensive and then they cannot be measured.

In the following table the results of repeated injections in an animal are recorded with the date of the experiments. In all cases the serum was diluted by adding four parts of 0.8 per cent sodium chloride solution to one part of serum. According to the intensity of previous reactions 0.05 or 0.1 cc. of this diluted serum was injected as indicated in the table. The reactions were measured 2, 4, 6, 8 and 24 hours after the injection, and a few times also after 10 hours. The side of the injection is noted also, R meaning that the injection was placed to the right of the median line, L, to the left.

The table shows that quite considerable differences may exist in the intensity of the reactions in the same animal at different times and, furthermore, in some cases it seems that one side may react more intensely than the other. For instance, in No. 1 the reactions on the right side were more extensive than those on the left. It is hardly feasible to give all our protocols in detail. But the notes taken in No. 1 illustrate that other factors besides the size of the infiltration may have to be taken in consideration under certain circumstances in comparing the intensity of two reactions. On February 27 the reaction on the left is described as very sharply defined, much elevated and firmly indurated, while on March 11 the larger reaction on the right side is characterized as fairly sharply defined with a rather diffuse swelling decidedly less elevated and less firmly indurated than that of February 27. The difference of intensity, therefore, between these two reactions is really not as marked as the measurements seem to indicate. Nevertheless, certain variations in the intensity of the reactions are observed at repeated injections in the same animal, a fact which is not surprising. This means

TABLE I
No. 1. 0.05 cc.

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TEN HOURS	TWENTY- FOUR HOURS
February 15, R.....	20.0	25.0	30.5	30.5	30.5	
February 18, L.....	17.0	20.5	20.0	20.0		26.0
February 22, R.....	20.5	31.5	31.5	27.5		21.5
February 27, L.....	18.5	19.5	21.5	20.5	37.0	22.5
March 11, R.....	20.0	36.5	37.0	39.5		34.0
March 15, L.....	17.3	23.0	22.0	23.0		23.0

No. 2. 0.05 cc.

February 15, R.....	21.0	26.5	25.5	25.0	18.0	17.0
February 18, L.....	18.5	20.0	21.0	27.5		19.0
February 22, R.....	16.0	24.5	26.5	22.0		15.0
February 27, L.....	13.5	24.0	24.0	21.0		20.0
March 11, R.....	16.5	20.5	22.0	20.5		13.5
March 15, L.....	19.5	19.5	19.5	19.5		0.0

No. 3. 0.05 cc.

February 15, R.....	31.0	31.5	35.5	35.5	28.5	24.0
February 18, L.....	21.5	26.5	27.0	23.5	34.0	31.0
February 22, R.....	23.0	31.0	36.5	36.5		14.5
February 27, L.....	16.5	24.0	24.0	23.5		18.5
March 11, R.....	21.0	38.0	38.0	38.0		killed; very sick

No. 4. February 27. 0.05 cc., later 0.1 cc.

February 27, L.....	18.5	19.5	21.5	20.5	29.5	22.5
March 11, R.....	19.5	24.5	29.5	29.5		27.5
March 15, L.....	22.5	22.0	23.0	24.0		23.0
March 23, R.....	15.5	23.5	25.0	20.5		18.5

No. 5. 0.05 cc.

February 15, R.....	25.5	26.0	26.5	26.5	26.5	25.0
February 18, L.....	20.5	25.0	27.0	29.0	27.5	28.5
February 22, R.....	20.5	26.5	28.0	27.0		19.5
February 27, R.....	15.0	22.0	22.0	20.5		18.5
March 11, R.....	22.0	27.5	27.5	27.5		25.0
March 15, L.....	16.0	20.5	21.5	21.5		19.5

TABLE I—CONTINUED

No. 7. February 15 to March 23, 0.05. March 28, 0.1 cc.

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TEN HOURS	TWENTY- FOUR HOURS
February 15, R.....	19.0		22.5	23.0	23.0	17.5
February 18, L.....	17.0	20.5	23.0	24.5		20.5
February 22, R.....	18.0	27.5	27.5	25.5		+
February 27, L.....	16.0	20.0	22.5	22.5		17.5
March 11, R.....	17.0	19.5	17.5	17.5	+	+
March 15, L.....	20.5	23.5	23.5	23.5		17.5
March 23, R.....	15.5	15.5	17.0	15.5		13.5
March 28, L.....	19.0	21.0	25.5	27.0		20.0

No. 8. 0.05 cc.

February 15, R.....	24.0	28.5	29.5	28.5	28.5	25.0
February 18, L.....	19.0	24.5	25.0	27.0		28.5
February 22, R.....	19.0	27.0	27.0	23.5		22.5
February 27, L.....	15.5	21.5	25.0	25.0		20.0
March 11, L.....	15.5	22.0	22.0	22.0	22.0	22.0
March 15, L.....	18.5	22.5	26.0	26.0		20.0

No. 9. 0.1 cc.

March 11, R.....	21.0	28.5	28.5	29.5	29.5	28.0
March 15, L.....	26.0	27.0	28.0	28.5		
March 23, R.....	20.0	27.5	29.5	29.5		25.5

No. 12. February 15 to March 23. 0.05 cc., March 28. 0.1 cc.

February 15, R.....	12.5	17.5	19.5	20.0	20.0	19.0
February 18, L.....	13.5	18.0	23.0	25.0		21.0
February 22, R.....	18.0	19.5	22.0	24.5		18.0
February 27, L.....	13.0	16.5	19.0	19.5		20.0
March 11, R.....	12.5	16.0	19.5	19.5	19.0	14.0
March 15, L.....	19.0	20.5	21.5	21.5		16.5
March 23, R.....	14.0	15.5	19.5	19.5		16.5
March 28, L.....	22.5	26.0	28.5	31.0		26.0

No. 17. 0.05 cc.

February 15, R.....	Pronounced haemorrhage.			Diffuse slight swelling.		
February 18, L.....	16.0	24.0	24.5	25.0		22.5
February 23, R.....	18.0	27.5	27.0	24.0		19.0
February 27, L.....	22.5	28.0	28.0	28.5		19.5
March 11, R.....	18.0	22.5	23.5	23.5	23.5	22.5
March 15, L.....	+	26.0	26.5	27.5		22.5

TABLE I—CONTINUED

No. 19. 0.1 cc.

		TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TEN HOURS	TWENTY- FOUR HOURS
March	11, R.	16.5	19.0	19.0	17.0	17.0	16.5
March	15, L.	27.5	27.0	28.5	28.5		23.5
March	23, R.	18.0	23.5	24.5	18.5		16.5

that the differences of the reactions must be striking in order to establish an influence of various procedures like the administration of a drug.

As a rule the reactions reach their maximal development within six to eight hours after the injection. After this time a retrogression sets in in most cases. This retrogression is more marked with regard to the degree of infiltration than to the size, so that the measurements after twenty-four hours are less representative than at the earlier stages. In some cases there is no appreciable retrogression in size nor degree of infiltration within twenty-four hours and in rare instances the intensity of a reaction increases after eight hours. Such occurrences are not constant in a given animal. Certain features of a reaction, on the other hand, may be rather characteristic for a certain animal. So, for instance, the reactions of No. 17 throughout the experiments were associated with pronounced haemorrhages.

Rabbit No. 3 had lost considerable weight during the period of observation. On the day of the last experiment it had taken its food well and did not show any untoward symptoms. The next morning it was found in its cage with very labored breathing and it had violent convulsions. The animal was killed with chloroform. The injection made within twenty-four hours before the onset of these severe symptoms was followed by a reaction certainly not less in intensity than the preceding ones. We shall have occasion to return to this observation.

THE INFLUENCE OF DILUTIONS OF THE SERUM ON THE INTENSITY
OF THE INTRACUTANEOUS REACTION

A number of experiments was undertaken, using different dilutions of serum. These experiments brought uniform results, which shall be illustrated by a few examples. For instance, Nos. 1, 2, 17, and 19, having been used in the meantime for a number of other experiments, received on June 30 0.1 cc. serum in the usual dilution of 1 : 5 simultaneously on the right and left side of the abdomen, and on July 7 0.1 cc. undiluted serum. The results are recorded in the following table:

TABLE II

		TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
1	{ June 30.....	14.5	16.5	18.5	18.3
	{ July 7.....	19.5	22.8	25.5	30.3
2	{ June 30.....	15.0	17.5	17.5	0.0
	{ July 7.....	24.0	Running together		
17	{ June 30.....	14.5	18.0	21.8	21.8
	{ July 7.....	18.5	28.8	29.5	30.5
19	{ June 30.....	14.8	15.3	15.3	0.0
	{ July 7.....	17.3	22.8	27.5	29.5

The measurements represent the average of the simultaneous reactions on the right and left side of the abdomen. This method, giving the injection simultaneously on both sides, was employed frequently in experiments to be recorded later, where the controls will be considered. In No. 2 the reactions were running together, therefore the measurements are not given. It is to be remarked that these animals had been used previously for a number of different experiments and that the intensity of the reactions had gradually diminished.

Rabbits Nos. 22, 30, 32, 34, 35, and 37 received July 7 on the right side of the abdomen an injection of 0.1 cc. serum diluted 1 : 10 and on the left 0.1 cc. serum diluted 1 : 66.6. Table III shows the result.

The size of the reactions in tables II and III represents very well the intensity of the reactions, for in every instance the smaller size was associated with a lesser degree of infiltration.

TABLE III

July 7

		TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
22	{ R.....	20.5	26.0	27.0	20.0
	{ L.....	15.5	16.5	16.5	0.0
30	{ R.....	20.5	25.0	29.0	27.0
	{ L.....	13.5	18.0	18.0	0.0
32	{ R.....	19.0	20.5	22.5	19.0
	{ L.....	13.0	15.0	16.5	12.0
34	{ R.....	19.0	25.0	26.5	25.0
	{ L.....	12.0	14.0	14.5	12.0
35	{ R.....	17.0	21.5	23.0	22.0
	{ L.....	11.5	13.0	14.0	14.0
37	{ R.....	20.0	24.0	25.5	26.5
	{ L.....	13.5	17.0	18.0	15.5

Averages

R.....	19.3	23.7	25.6	23.3
L.....	13.2	15.6	16.3	8.9

Tables II and III show clearly that the intensity of the intracutaneous reaction of a given animal is dependent on the amount of serum injected. Curve A, based on the average measurements of table III, gives a graphical illustration of these results. The ordinates of this curve and those to follow later represent the measurements—that is the intensity of the reactions, while the time after the injection is marked on the abscissa. A close analogy exists between these results and those obtained by von Pirquet (13) with the “early reaction” in cow-pox vaccination and the cutaneous tuberculin reaction (12), as well as with those of Amberg (14) with the cutaneous trichophytin reaction. In all these cases the intensity of the reaction diminishes with the diminution of the introduced allergen. A number of experiments had been made testing the effect of different dilutions of serum, using on one side 0.01 cc. undiluted, on the other the same volume of diluted serum. The results were the same as in the experiments just reported. In one series, however, comprising a set of four animals (Nos. 2, 3, 5 and 12) 0.01 cc. undiluted serum was

injected on one side of the abdomen and 0.1 cc. of a serum diluted ten times with saline, on the other. That is, the same amount of serum was given, but in different volume. In every case the reactions with the diluted serum were quite markedly more intense than with the undiluted serum. We did not follow this factor any further. The results indicate the possibility of an influence of the concentration of the allergen, so that the same amount of allergen given in lesser concentration elicits a more intense reaction. It is possible that a better distribution of the allergen enters here in consideration. We took cognizance of this possibility throughout our work.

THE INFLUENCE OF INTRAVENOUS INJECTIONS OF LARGE DOSES OF SERUM ON THE INTRACUTANEOUS REACTION

The influence of intravenous injections of relatively large doses of serum on the intracutaneous reaction was made the subject of a few experiments. It is known that a guinea-pig sensitized to a protein, receiving a second injection of this protein in a dose not leading to death, becomes refractory for a time to further injections (15). Moss and Brown (11) studying the effect of larger doses of horse serum on the intracutaneous reaction of rabbits sensitized to horse serum found a diminution or even suppression of the intracutaneous reaction. Our experiments were made with rabbits Nos. 1, 2, 5, 8, and 17. These animals received intravenously amounts of serum indicated in table IV, and $1\frac{1}{2}$ to $2\frac{1}{2}$ hours later an intracutaneous injection of 0.05 cc. serum diluted 1 : 5. The table contains the averages of the results obtained in the previous experiments recorded in table I. Next the results of the experiments with the intravenous injections of serum are given to which are joined results obtained about one week later.

TABLE IV

No. 1

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Average of 1.....	18.9	26.0	27.1	26.8	26.1
March 21, 6.0 cc. serum.....	11.0	11.5	11.5	10.0	0.0
March 30.....	15.0	25.0	27.0	24.5	19.0

No. 2

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Average of 2.....	17.5	22.5	23.1	22.6	12.4
March 21, 6.0 cc. serum.....	14.5	17.5	18.0	14.0	9.5
March 30.....	15.5	25.5	29.0	26.5	+

No. 5

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Average of 5.....	19.9	24.6	25.4	25.3	22.7
March 23, 5.0 cc. serum.....	14.0	16.5	18.5	18.5	15.0
March 30.....	15.0	21.5	24.5	24.5	17.0

No. 8

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Average of 8.....	18.6	24.3	25.8	25.3	23.0
March 23, 6.0 cc. serum.....	15.0	19.0	18.5	17.5	15.0
March 30.....	18.5	22.5	27.0	27.0	+

No. 17

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Average of 17.....	18.6	25.6	25.9	25.7	21.2
March 23, 10.0 cc. serum.....	12.5	10.5	9.0	8.5	+
March 30.....	16.0	23.0	23.0	23.0	20.5

The results of table IV may be summarized briefly, calculating the averages of all the animals as follows:

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Before administration of serum..	18.7	24.6	25.5	25.1	21.1
After administration of serum..	13.4	15.0	15.1	13.7	7.9

Curve *B* based on these figures depicts the result graphically.

In these experiments again the degree of infiltration showed a decided diminution with the diminution of the reaction area.

It is evident that the intravenous injection of larger doses of serum in sensitized animals diminishes the intensity of the intracutaneous reaction. This influence is not lasting, since after an interval of seven to nine days the effect is lost. Rabbits Nos. 2 and 17 did not show any symptoms whatever after the intravenous injection of the serum. No. 5 was very quiet for a short time. No. 1 was very listless for about thirty minutes and had a very rapid respiration. These symptoms then subsided rapidly while in the course of the day a few diarrhoic stools were passed. No. 8 showed the same symptoms though less pronounced. It was tried to obtain about 0.05 cc. blood from the ear vessels of Nos. 1 and 8 before and after the administration of the serum. Before the injection there was no difficulty, but after the injection it was impossible to obtain the small amount of blood required for some time in spite of application of heat or rubbing. All the ear vessels appeared empty (13). The same difficulty had been encountered before with other sensitized rabbits after the intravenous administration of large doses of serum. A few non-sensitized animals, having received 5 cc. serum intravenously, did not present this difficulty. It is possible that the behavior noted is connected with a fall of pressure in the general circulation observed on intravenous injections of allergen in sensitized animals, and which occurs in the rabbit also (17).

THE INFLUENCE OF IODOXYBENZOIC, IODBENZOIC AND BENZOIC ACID AND SODIUMCYANIDE ON THE INTRACUTANEOUS REACTION

A series of animals, Nos. 4, 7, 9, 12, and 19, received in the ear vein 10 cc. of $\frac{N}{20}$ iodoxybenzoic acid in the form of its sodium salt in a carefully neutralized solution. This was followed immediately by an intracutaneous injection of 0.1 cc. serum diluted 1 : 5. The measurements of the previous reactions are given in table I. In table V the average of these previous measurements are given in Nos. 4, 9, and 19. In Nos. 7 and 12, where the first

injections had been made with 0.05 cc. diluted serum, the measurements of the reactions preceding this experiment, when 0.1 cc. diluted serum had been used, are given. One or two days after the administration of iodoxybenzoic acid another intracutaneous test was made and the results are recorded also.

TABLE V

No. 4.

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Average of 4 (March 11, 15, 23)...	19.2	23.2	25.8	24.7	23.0
March 28, Iodoxybenzoic acid...	13.5	12.5	9.5	+	0.0
March 30.....	20.0	24.0	25.5	25.5	22.2

No. 7

March 28.....	19.0	21.0	25.5	27.0	20.0
April 3, Iodoxybenzoic acid.....	0.0	0.0	0.0	0.0	0.0
April 4.....	14.0	19.5	18.0	18.0	16.5

No. 9

Average of 9.....	22.3	27.7	28.7	29.2	26.8
March 28, Iodoxybenzoic acid...	8.5	11.0	11.0	11.0	8.0
March 30.....	Moribund. Four hours after injection, dead, reaction negative.				

No. 12

March 28.....	22.5	26.0	28.5	31.0	26.0
April 3, Iodoxybenzoic acid.....	13.0	14.0	14.5	14.5	22.5
April 4.....	12.0	17.5	27.5	27.5	27.5

No. 19

Average of 19.....	20.7	23.2	24.0	21.3	18.8
March 28, Iodoxybenzoic acid...	10.0	8.0	8.0	8.0	
March 30.....	18.5	24.5	25.0	25.0	18.5

Calculating the averages from this series we have:

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Before iodoxybenzoic acid.....	20.7	24.3	26.5	26.6	22.9
After iodoxybenzoic acid.....	9.0	9.1	8.6	6.7	6.1

Rabbit No. 12 showed a peculiar course of the reactions after the administration of the drug on April 3. Eight hours after the intracutaneous injection the reaction measured 14.5 mm., while after twenty-four hours on April 4, it had increased to 22.5, with a decided increase in the degree of infiltration and redness. During that day the reaction increased further in intensity measuring 25 mm. gradually diminishing from now on. The morning of April 4 another intracutaneous injection had been given and the further increase of the first reaction on that day is perhaps referable to this second injection.

The experiments with iodoxybenzoic acid were repeated several times. The method differed from that used in this series in as much as the animals received each time the intracutaneous injection on both sides of the abdomen and the measurements represent the average diameter. The possible influence of variations due to different placing of the injections is thereby also eliminated. This factor, though, is of very little importance here where the results are so striking. Table VI contains the average diameter of the reactions from two experiments of April 11 and 19, followed by the figures for April 25, when iodoxybenzoic acid had been administered in the same manner and strength as before. The animals serving for this series were Nos. 1, 4, 5, 7, 8, 12, 17, and 19.

TABLE VI

No. 1

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Average of 1 (April 11, 19).....	21.6	24.3	25.0		25.0
April 25, Iodoxybenzoic acid....	6.5	11.5	12.3	14.8	17.0

No. 4

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Average of 4 (April 11, 19).....	17.1	19.1	21.0		18.8
April 25, Iodoxybenzoic acid....	10.0	11.5	11.5	11.5	0.0

No. 5

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Average of 5 (April 11, 19).....	20.8	21.6	23.3		23.1
April 25, Iodoxybenzoic acid....	0.0	0.0	7.3	6.0	7.5

TABLE VI—CONTINUED

No. 7

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Average of 7 (April 11, 19).....	18.9	21.6	22.0		16.0
April 25, Iodoxybenzoic acid....	0.0	0.0	0.0	0.0	0.0

No. 8

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Average of 8 (April 11, 19).....	21.6	24.4	27.1		23.4
April 25, Iodoxybenzoic acid....	6.3	6.3	14.8	15.8	16.0

No. 12

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Average of 12 (April 11, 19).....	18.3	23.1	26.3		25.9
April 25, 15.0 Iodoxybenzoic acid	0.0	0.0	0.0	10.0	

No. 17

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Average of 17 (April 11, 19).....	18.0	25.5	26.8		26.9
April 25, Iodoxybenzoic acid....	0.0	13.3	15.5	15.5	16.8

No. 19

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Average of 19 (April 11, 19).....	18.9	19.8	21.1		18.1
April 25, Iodoxybenzoic acid....	12.8	13.5	13.5	9.5	0.0

The average diameters of all the reactions before and after the administration of the sodium iodoxybenzoate are:

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY- FOUR HOURS
Before.....	19.7	22.4	24.1		21.9
After.....	4.0	7.0	9.3	10.1	8.3

Two animals of this series, Nos. 1 and 8, showed a late increase in the intensity of the reaction like No. 12 in table IV.

The following experiments, conducted as those just reported, were made with a new series of animals, with exception of No. 8. Nos. 23, 25, and 31 had not received any other injections of drugs. Nos. 22, 29, and 30 had received benzoic acid on June 24, when Nos. 32, 34, 35, and 37 had received iodbenzoic acid intravenously.

No. 8 had a more checkered career. Table VII gives the average diameters of the reactions antedating the administration of 10 cc. $\frac{N}{20}$ iodoxybenzoic acid as sodium salt, the diameters of the intracutaneous reaction immediately following it and those obtained two or three days later.

TABLE VII

No. 23

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
Average of No. 23 (June 8, 13, 19).....	14.7	19.0	21.2	20.6
June 24, Iodoxybenzoic acid.....	0.0	0.0	0.0	0.0
June 26.....	15.0	20.3		15.5

No. 25

Average of No. 25 (June 8, 13, 19).....	14.7	17.1	18.7	16.0
June 24, Iodoxybenzoic acid.....	0.0	0.0	0.0	0.0
June 26.....	15.3	19.3		14.0

No. 31

Average of No. 31 (June 8, 19).....	12.3	16.2	18.9	6.5
June 24, Iodoxybenzoic acid.....	0.0	0.0	0.0	0.0
June 26.....	13.3	16.3		0.0

No. 8

Average of No. 8 (April 11, 19, May 31, June 13, 19).....	19.7	23.6	25.4	20.8
June 30, Iodoxybenzoic acid.....	0.0	0.0	13.0	14.3
July 3.....	died			

No. 22

Average of No. 22 (June 8, 13, 19).....	16.2	21.0	21.6	17.5
June 30, Iodoxybenzoic acid.....	5.8	6.5	14.5	11.3
July 3.....	20.5	26.3	28.5	

No. 29

Average of No. 29 (June 8, 13, 19).....	18.6	22.8	27.4	25.5
June 30, Iodoxybenzoic acid.....	6.8	6.5	15.0	died 19.0

No. 30

Average of No. 30 (June 8, 13, 19).....	21.3	26.4	31.0	27.8
June 30, Iodoxybenzoic acid.....	12.3	14.0	15.0	16.3
July 3.....	19.0	24.5	28.3	

TABLE VII—CONTINUED

No. 32

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
Average of No. 32 (June 8, 13, 19).....	16.8	20.2	22.8	6.1
June 30, Iodoxybenzoic acid.....	0.0	0.0	0.0	0.0
July 3.....	20.0	27.0	29.8	

No. 34

Average of No. 34 (June 8, 13, 19).....	18.7	24.3	28.5	32.4
June 30, Iodoxybenzoic acid.....	0.0	0.0	15.0	22.5
July 3.....	20.5	25.3	26.3	

No. 35

Average of No. 35 (June 8, 13, 19).....	17.3	18.5	18.9	19.0
June 30, Iodoxybenzoic acid.....	12.3	12.0	12.8	12.8
July 3.....	16.5	18.5	20.8	

No. 37

Average of No. 37 (June 8, 13, 19).....	22.0	29.4	33.5	31.8
June 30, Iodoxybenzoic acid.....	14.3	17.0	21.3	23.0
July 3.....	24.3	30.0	34.3	

The average diameters of all the reactions before and after the administration of sodium iodoxybenzoate are:

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
Before.....	17.3	21.7	24.4	20.4
After.....	4.7	5.1	9.7	10.8

In this series we see again a delayed increase of intensity in Nos. 29, 34, and less in 37. In No. 35 a few cubic centimeters of the iodoxybenzoic acid solution escaped in the subcutaneous tissue.

In all the experiments the diminution of the size of the reaction following the administration of the drug was associated with a very decidedly lessened degree of infiltration. The result of all the experiments is very striking. The intravenous administra-

tion of iodoxybenzoic acid as sodium salt in a carefully neutralized solution always diminishes the intracutaneous reaction very markedly, suppressing it entirely in some cases. This effect is not lasting, for the intensity of intracutaneous reactions obtained a few days after the administration of the drug do not, on the whole, differ very materially from those preceding its administration.

Curve *C* is intended as a graphic illustration of this effect of the administration of iodoxybenzoic acid using the average diameters of all the reactions recorded in tables V, VI and VII, as follows:

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
Before administration of sodium iodoxybenzoate.....	18.8	22.5	24.6	21.5
After administration of sodium iodoxybenzoate.....	5.1	6.6	9.4	9.0

Iodoxybenzoic acid has the formula $\text{C}_6\text{H}_4 \begin{array}{l} \diagup \\ \text{I}=\text{O} \\ \diagdown \text{O} \\ \text{COOH} \end{array}$. The

question arose whether the oxygen contained in the molecule in combination with the iodine is likely to be responsible for the action of the iodoxybenzoic acid in our experiments, or whether the iodine or the benzoic acid contained in the molecule play a rôle. Control experiments were made with iodbenzoic acid of

the formula $\text{C}_6\text{H}_4 \begin{array}{l} \diagup \text{I} \\ \diagdown \text{COOH} \end{array}$ and benzoic acid.

The conditions of these experiments were the same as in tables VI and VII, only substituting in one series iodbenzoic, in the other benzoic acid for the iodoxybenzoic acid. In either case 10 cc. of $\frac{N}{20}$ acid was given intravenously in the form of the sodium salt in carefully neutralized solutions. Table VIII contains the results of these experiments. Rabbits Nos. 1, 2, 8, 17, 19, 32, 34, 35, and 37 received iodbenzoic acid, rabbits Nos. 1, 2, 8, 17, 19, 22, 29, and 30 benzoic acid.

TABLE VIII
*Iodbenzoic Acid**No. 1*

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
Average of 1 (April 11, 19, May 31).....	19.5	23.2	24.5	24.5
June 8, Iodbenzoic acid.....	13.8	17.8	22.5	24.3
June 13.....	14.8	18.3	18.3	18.3

No. 2

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
Average of 2 (April 11, 19, May 31).....	18.9	22.1	22.3	18.7
June 8, Iodbenzoic acid.....	14.8	16.0	17.8	15.5
June 13.....	14.5	19.5	19.5	0.0

No. 8

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
Average of 8 (April 11, 19, May 31).....	21.1	24.8	26.7	23.1
June 8, Iodbenzoic acid.....	12.5	21.5	25.8	22.5
June 13.....	15.3	19.5	21.3	14.8

No. 17

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
Average of 17 (April 11, 19, May 31).....	18.9	24.9	25.9	24.8
June 8, Iodbenzoic acid.....	12.5	20.3	21.5	21.5
June 13.....	13.8	16.8	17.5	18.8

No. 19

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
Average of 19 (April 11, 19, May 31).....	18.3	22.1	23.3	19.3
June 6, Iodbenzoic acid.....	11.8	15.5	17.3	0.0
June 13.....	16.5	18.5	18.5	12.5

No. 32

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
Average of No. 32 (June 8, 13, 19).....	16.8	20.2	22.8	6.1
June 24, Iodbenzoic acid.....	19.0	22.8	26.8	13.5

No. 34

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
Average of No. 34 (June 8, 13, 19).....	18.7	24.3	28.5	32.4
June 24, Iodbenzoic acid.....	20.0	33.3	36.0	38.5

No. 35

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
Average of No. 35 (June 8, 13, 19).....	17.3	18.5	18.9	19.0
June 24, Iodbenzoic acid.....	16.0	20.8	21.8	20.8

TABLE VIII—CONTINUED

No. 37

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
Average of No. 37 (June 8, 13, 19).....	22.0	29.4	33.5	31.8
June 24, Iodobenzoic acid.....	21.0	31.0	32.8	31.5

*Benzoic Acid**No. 1*

Average of No. 1 (April 11, 19, May 31, June 13, 19).....	17.6	21.1	21.8	21.5
June 24, Benzoic acid.....	15.8	20.8	22.5	17.8

No. 2

Average of 2 (April 11, 19, May 31, June 13, 19).....	17.4	20.8	21.0	11.2
June 24, Benzoic acid.....	16.0	19.8	18.0	0.0

No. 8

Average of 8 (April 11, 19, May 31, June 13, 19).....	19.7	23.6	25.4	21.0
June 24, Benzoic acid.....	20.8	28.5	31.0	25.3

No. 17

Average of 17 (April 11, 19, May 31, June 13, 19).....	16.0	21.3	22.4	21.5
June 24, Benzoic acid.....	14.3	22.5	25.8	28.5

No. 19

Average of 19 (April 11, 19, May 31, June 13, 19).....	17.5	20.9	21.6	17.3
June 24, Benzoic acid.....	15.0	17.0	17.8	10.5

No. 22

Average of No. 22 (June 8, 13, 19).....	16.2	21.0	21.6	17.5
June 24, Benzoic acid.....	18.0	21.0	24.8	15.3

No. 29

Average of No. 29 (June 8, 13, 19).....	18.6	22.8	27.4	25.5
June 24, Benzoic acid.....	20.8	28.5	32.0	32.8

No. 30

Average of No. 30 (June 8, 13, 19).....	21.3	26.4	31.0	27.8
June 24, Benzoic acid.....	26.0	32.3	35.5	32.0

The total average diameters of the reactions before and after the administration of iodbenzoic acid are:

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
Before.....	19.1	23.3	25.2	22.2
After.....	15.7	22.1	24.7	20.9

The experiments with benzoic acid may be summarized as follows:

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
Before.....	18.0	22.2	24.0	20.4
After.....	18.3	23.8	25.9	20.3

The experiments with iodbenzoic acid were made in two series. In the first with animals Nos. 1, 2, 8, 17, and 19, it seems on first sight, that the drug exercised a diminishing effect on the reaction. But the reactions obtained subsequently were also less intense than the average of the reactions before the administration of iodbenzoic acid. Furthermore, in rabbits Nos. 32, 34, 35, 37, animals more recently sensitized and not used before for other experiments, certainly no such effect is noticeable. It may be stated that neither iodbenzoic nor benzoic acid exercise an appreciable diminishing effect on the intracutaneous reaction.

Without any doubt there exists a great difference between the effect of iodoxybenzoic acid and that of iodbenzoic and benzoic acid, when these substances are administered in equimolecular concentrations.

We gratefully acknowledge our indebtedness to Dr. A. S. Loevenhart who furnished us the iodoxy and iodbenzoic acid. Loevenhart (18) introduced the iodbenzoic acid series in pharmacology, studying the action of iod, iodoso and iodoxybenzoic acid. His investigations leave hardly any doubt that the oxygen of iodoxybenzoic acid—readily given off in the test tube—is given off in the organism in a physiologically active form. From all the data at our command it seems that the oxygen of the iodoxybenzoic acid is the determining factor for its activity in our experi-

ments. Therefore, it is very probable that the effects of this substance are primarily due to its influence on oxidative processes. This assumption was the reason why we choose this compound for our experiments. It may be mentioned here that Manwaring (19) also figured with the possibility that oxidation processes play a rôle in the anaphylactic reactions, having found that the elimination of the liver from the circulation suppresses the fall of blood pressure on reinjection of sensitized animals, results strikingly substantiated by Voegtlin and Bernheim (20) using a better method of experimentation.¹

In any effort to explain the effect of iodoxybenzoic acid on the intracutaneous reaction, first the question arises whether this effect may not be due primarily to a general toxic effect so that the intoxicated animal can no longer respond in its usual manner to the intracutaneous injection of the serum.

This possibility suggests itself from the fact that in six of our twenty-four experiments the animals died. All animals receiving sodium iodoxybenzoate showed some transitory symptoms. Most of the animals were rather quiet for some time after the injection, sometimes after a very transitory period of excitement. As a rule the animals recovered rapidly and after a period of one or two hours no appreciable symptoms were noted. Frequently there was some respiratory distress of rather short duration and not very pronounced. In some cases the injection seemed to be accompanied with pain, particularly when some of the solution escaped in the tissues. When this happened a more or less extensive necrosis followed. After the initial symptoms had subsided the animals did not betray any further symptoms sufficiently marked to be noted, so that the fatal result was rather surprising, the more so as in several preliminary experiments with non-sensitized rabbits we did not have any fatal results even with larger doses. Only one rabbit died with 10 cc. sodium iodoxybenzoate. This animal died immediately after a rapid injection. In all other cases the injections were made slowly. However, our control experiments with sodium iodoxybenzoate are

¹ Friedberger and Gröber are inclined to attribute the results of Manwaring to a lack of complement due to the elimination of the liver from the circulation.

not sufficient' in number to warrant definite conclusions with regard to its toxicity. Iodobenzoic and benzoic acid did not elicit any symptoms. Another factor may have to be taken in consideration. Heilner (21) as well as Davidsohn and Friedemann (22) observed symptoms in sensitized animals on the administration of sodium chloride which did not occur in non-sensitized animals. Similarly sensitized animals might be more susceptible to sodium iodoxybenzoate than nonsensitized animals.

A somewhat more detailed description of our fatal cases does not seem out of place.

Rabbit No. 9 of table V died the second day after the administration of the drug. On the day of the injection and the day following no special symptoms were noted, the animal being lively and taking its food well. No. 12 in table VI, having received previously 10 cc. iodoxybenzoic acid, showing the late increase of the intracutaneous reaction as noted above, received now 15 cc. apparently with a more striking effect. The morning following the injection this animal was found dead in its cage. The evening before it had been lively, running around, eating well and with its fur in good condition. No. 4 of this series died in the afternoon of the day following the injection without having presented any warning signs the same forenoon, and No. 5 was found dead on the morning of the second day after the injection, having been apparently well the day preceding its death. No. 8 of table VII was found dead in rigor mortis the second morning after the injection. For about six hours after the injection the respiration was rather rapid and somewhat labored, the animal sitting in its cage rather quiet. The next day these symptoms had subsided. No. 29 was found dead, still warm, at 11 a.m. on the day following the injection. On the day of injection this animal also remained rather quiet and showed for several hours somewhat labored breathing, while the next morning some diarrhoic stool was found in the cage. At eight in the morning of this day it was found running around free in the laboratory without showing any symptoms. The last two animals were the most affected by the administration of sodium iodoxybenzoate. No. 29 is of special interest. Six hours after the in-

jection the average diameter of its reaction was 15 mm., it was not measured again before its end. When measured the diameter had increased to 19 mm. with a concomitant increase in degree of infiltration. The autopsy in the cases just described showed some oedema of the lungs and hemorrhagic areas in the lungs, from pin-head to pea-size; rarely larger. In some cases they were fairly numerous, in others only a few were found and in none was the greater part of the lungs affected. No other macroscopical lesions were found.

That a general intoxication may influence the intracutaneous reaction appears probable from a few experiments with hydrocyanic acid. On May 9 rabbits 7, 17, and 19 received on one side of the abdomen 0.1 cc. of serum diluted 1 : 5 with saline and on the other 0.1 cc. serum diluted 1 : 5 with 1 per cent hydrocyanic acid, while in a third place 0.1 cc. 1 per cent hydrocyanic acid was given as control. All these animals had violent convulsions and very severe dyspnoea. No. 7 died, Nos. 17 and 19 began to recover slowly after a period of about one hour. In these animals the reactions were strikingly less intense than previously. At the same time Nos. 1, 2, and 3 received the same injections with exception of the control. Here no appreciable symptoms occurred and the reactions, particularly those with the serum diluted with saline, did not show any marked differences from those obtained previously. But here we have a very definite severe intoxication and while the reactions were markedly diminished, the effect was not as pronounced as the effect of iodoxybenzoic acid in the same animals.

Furthermore, the reactions of the animals which died were not more affected than those of the others, and No. 12 in table VI, Nos. 8 and 29 in table VII showed a late increase of their reactions. Here we may refer to our observation of rabbit 3 in table I, where the reaction certainly did not show any diminution although the next morning following the intracutaneous injection it was found with the severest symptoms. A few other animals where the reactions were not measured, died the day following the intracutaneous injection or within a few days following it without an appreciable diminution of the reactions.

The sum total of our observations is not in favor of the view that a general toxic effect is vitally concerned in the diminution of the intracutaneous reaction due to the administration of sodium iodoxybenzoate.

We tried to approach this problem by a different method, whereby the rabbits received on one side of the abdomen serum diluted with saline, on the other serum diluted with sodium iodoxybenzoate solution in the usual proportion. The serum diluted with the latter remained perfectly clear. A third injection of 0.1 cc. sodium iodoxybenzoate solution was given as control. In the first series of April 4 the strength of this solution was $\frac{N}{20}$, in the second of May 26 $\frac{N}{10}$. In table IX "R" designates the reactions obtained with the serum diluted with sodium iodoxybenzoate, "L" those with serum diluted with saline and "C" the control with sodium iodoxybenzoate.

TABLE IX

*April 4**No. 1*

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY-FOUR HOURS
R.....	12.5	21.5	24.5	24.5	22.0
L.....	16.5	22.0	22.5	22.5	19.5
C.....	12.5	16.0	16.5	16.5	13.0

No. 2

R.....	16.0	19.0	22.0	19.0	14.0
L.....	19.0	23.5	25.5	26.0	17.5
C.....	14.5	14.5	14.5	14.5	8.5

No. 5

R.....	15.0	22.0	24.5	24.5	22.0
L.....	13.5	24.5	28.5	28.5	26.5
C.....	9.0	0.0	0.0	0.0	0.0

No. 8

R.....	15.5	21.5	24.5	24.5	18.5
L.....	19.5	23.5	28.0	28.0	25.0
C.....	15.0	17.0	17.0	17.0	11.5

TABLE IX—CONTINUED

No. 17

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY-FOUR HOURS
R.....	0.0	19.0	21.0	21.0	19.0
L.....	22.0	31.5	32.5	32.5	30.0
C.....	0.0	0.0	0.0	0.0	0.0

*May 26**No. 1*

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
R.....	13.0	19.0	24.0	23.0
L.....	18.5	26.0	30.5	30.0
C.....	13.5	16.0	19.0	19.5

No. 2

R.....	16.5	25.5	24.5	19.0
L.....	23.0	26.0	25.0	22.5
C.....	13.5	19.5	23.0	20.0

No. 8

R.....	15.0	22.0	25.5	22.5
L.....	20.0	26.5	28.5	27.0
C.....	10.5	16.5	18.0	18.5

No. 17

R.....	0.0	16.5	22.5	18.5
L.....	20.5	23.5	24.5	24.5
C.....	11.5	12.0	14.0	13.5

No. 19

R.....	0.0	16.5	22.5	18.5
L.....	20.5	23.5	24.5	24.5
C.....	11.5	12.0	14.0	13.5

The total average diameter of the reactions on April 4 with animals Nos. 1, 2, 5, 8, and 17 and $\frac{N}{20}$ sodium iodoxybenzoate:

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY-FOUR HOURS
R.....	11.8	20.6	23.3	24.7	19.1
L.....	18.1	25.0	27.4	27.5	23.7
C.....	10.2	9.5	9.6	9.6	6.6

and May 26 with animals 1, 2, 8, 17, and 19, and $\frac{N}{10}$ sodium iodoxybenzoate:

	TWO HOURS	FOUR HOURS	SIX HOURS	EIGHT HOURS	TWENTY-FOUR HOURS
R.....	12.6	20.3	24.7		20.3
L.....	22.6	27.9	29.5		25.7
C.....	12.9	16.4	18.4		16.3

These latter figures formed the basis for the construction of Curve *D*, where the upper curve represents the reactions on the left, the middle curve those on the right, and the lower the control.

With exception of No. 1, April 4, the size of the reactions agreed well with the degree of infiltration. In No. 1 during the first four hours the reactions on the left with serum diluted with saline exceeded that on the right with serum diluted with sodium iodoxybenzoate and the difference in intensity after four hours was greater than indicated by the size. After this time the reaction on the right spread more, while throughout the period of observation the elevation, redness, and induration on the left was so decidedly greater as to leave no doubt that this was the more intense reaction. The control with sodium iodoxybenzoate alone produced in most cases a swelling, etc., very similar to the other reactions, ending mostly in a superficial necrosis. In several cases the control after twenty-four hours differed very little from the reactions with serum diluted with the drug. In spite of this fact it is evident that the sodium iodoxybenzoate exercised a marked inhibitory effect on the reactions. This effect was most

marked after two and four hours. Later the difference between the reactions on either side was much less pronounced. It is questionable whether it is permissible to subtract the results obtained with the controls from those obtained with the serum diluted with the sodium iodoxybenzoate, a procedure which would make the inhibiting effect of the drug much greater.

In order to establish this inhibiting effect of our substance a number of control experiments had to be conducted, comparing the intensity of intracutaneous reactions on both sides of the abdomen on simultaneous injections. As in the cases where one injection was given the same animal at different times here also it was noted that the reactions vary in intensity on different dates. Furthermore, the simultaneous injections need not always elicit reactions of about the same intensity. This occurs rather frequently and on the whole the differences between the simultaneous reactions are not as great as the difference between reactions obtained at different times. Sometimes the reaction on one side exceeds in size and degree of infiltration, etc., that of the other. Both reactions may have about the same size, but differ in degree of infiltration. Or the reaction on one side may cover a greater area while the degree of infiltration is decidedly less than on the other. It would lead us too far to describe all the variations noted in detail. It will suffice for our purpose to give the average diameters of the actual controls for our experiments, adding a few more detailed protocols. In our observations with the drugs the differences were sufficiently marked to overcome the variations which we encountered in our control experiments.

Two control experiments with animals Nos. 1, 2, 5, 8, and 17 serving for the experiments with sodium iodoxybenzoate on April 4 gave the following results:

		TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
April 11	{ R.	23.3	26.2	28.8	26.4
	{ L.	22.9	25.2	26.9	23.9
April 19	{ R.	18.2	21.5	22.4	21.3
	{ L.	18.3	21.3	22.1	20.9

A number of control experiments with animals Nos. 1, 2, 8, 17, and 19 serving for the experiments with sodium iodoxybenzoate on May 26 resulted as follows:

		TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
April 11	R.....	22.8	26.1	28.5	25.1
	L.....	22.5	24.5	26.6	22.6
April 19	R.....	17.7	21.2	21.9	20.5
	L.....	18.0	20.9	21.6	20.3
May 31	R.....	18.5	25.0	25.4	21.1
	L.....	18.1	22.8	24.2	22.3
June 13	R.....	14.9	18.6	19.3	12.9
	L.....	15.0	18.4	18.7	12.8
June 19	R.....	14.9	18.6	18.6	12.6
	L.....	16.7	19.2	19.3	14.2

We will give some results with simultaneous injections in more detail. The dose was always the same, 0.1 cc. serum diluted 1 : 5 with saline. Rabbits 21 to 42 were sensitized as follows: On April 14 these animals received 1.0 cc. horse serum in the ear vein; this dose was repeated on April 19 and 22. It may be remarked that one animal died immediately after the last injection, while another was paralyzed in the hind legs with a loss of control of the sphincters. This animal was killed later, having given a positive test on April 28, 30, and May 3. On April 28 the first intracutaneous test was given with undiluted serum repeated on April 30, May 3, 9, 12, 18, 21, 26, and 31, when all the surviving eighteen animals reacted with exception of one. June 8, 13, and 19, ten of these animals received the simultaneous injections on either side of the abdomen. On June 13, No. 31 was omitted by mistake. In these records some of the reactions on the left side are marked >, meaning that the degree of infiltration, redness, etc., exceeded definitely that of the right side, and <, signifying the opposite behavior. We may add here one of the records of animals Nos. 1, 2, 8, 17, and 19 of April 11.

The good agreement, particularly between the average diameters of the simultaneous reactions in our control experiments,

JUNE 8				JUNE 13				JUNE 19				APRIL 11			
TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY- FOUR HOURS
22 { R..... 14.5	22.0	23.5	20.5	16.5	21.0	21.0	16.5	18.5	20.5	22.0	16.5	1..... { 24.0	29.0	29.5	29.5
L..... 12.5	22.0	23.0	19.5	17.0	20.0	20.0	15.0	18.0	20.5	22.0	17.0	{ 22.5	23.5	25.5	25.0 >
23 { R..... 14.5	23.0	25.5	24.5	17.5	20.0	22.0	20.0	12.5	17.0	16.0	16.0	2..... { 19.5	24.0	27.0	18.5
L..... 14.0	19.0 <	24.0	23.8	15.0	17.0 <	20.0 >	20.0	14.5 >	18.0 >	18.5 >	18.5	{ 19.0	23.5	25.0	18.0
25 { R..... 14.0	17.0	19.0	16.5	17.0	19.5	20.5	16.5	14.5	15.5	17.5	13.5	8..... { 25.5	27.0	31.0	28.5
L..... 13.0	16.5	18.5 <	16.5 <	17.0	18.0	19.5	16.0	12.5	16.0	17.0	17.0	{ 26.0	27.0	31.5	25.0
29 { R..... 19.0	23.5	29.5	31.5	16.0	20.5	24.0	16.5	20.0	22.0	28.5	27.0	17..... { omitted	28.5	30.5	29.0
L..... 18.0	25.5 >	32.0 >	31.0	18.0	22.0 >	25.0 >	22.0 >	20.5 >	23.0 >	25.5 >	25.0 >	{ 26.0	27.5	26.5	26.5 >
30 { R..... 19.5	26.0	33.5	29.5	21.5	26.5	31.0	28.0	22.5	26.0	29.5	25.5	19..... { 22.0	22.0	24.0	20.0
L..... 21.0	27.0	34.5	29.0	20.5	26.0	29.5	26.5	22.5	26.5	28.0	28.0	{ 22.5	22.5	23.5	18.5
31 { R..... 13.5	17.5	20.5	0.0	20.5				10.5	14.5	17.0	12.0				
L..... 14.0	17.5	19.0	0.0					12.0	15.0	19.0	14.0				
32 { R..... 16.5	23.0	22.5	0.0	15.5	18.0	19.0	0.0	20.5	21.5	26.5	17.5				
L..... 16.0	20.5	27.0	0.0	15.0	18.0	19.0	0.0	17.0	20.0	22.5	19.0				
34 { R..... 16.0	24.0	32.0	36.5	22.0	27.5	30.0	29.5	20.5	24.5	27.5	31.0				
L..... 15.0	24.5	31.5	39.0	19.0	22.5	25.0 >	28.5	19.5 >	22.5 >	25.0 <	30.0				
35 { R..... 15.5	17.0	17.0	19.0	16.5	18.0	21.0	19.5	17.5	18.0	17.0	17.0				
L..... 19.0 >	19.5 >	19.5 >	21.5 >	18.0 >	20.5 >	21.0	19.5	17.0	18.0	17.5	17.5				
37 { R..... 19.5	33.5	37.5	31.5	19.0	23.5	27.5	25.5	25.5	30.0	33.5	32.0				
L..... 21.0	33.0	38.0	40.5	21.5	25.0	29.5	27.0	25.0	31.5	35.0	34.5				
Total..... R	16.3	22.7	26.1	21.0	18.0	21.6	19.0	18.3	21.0	23.4	20.3				
Average..... L	16.4	22.5	26.7	22.1	18.0	21.0	17.2	17.9	21.1	23.0	22.1				

as well as the uniform results with the local application of serum diluted with sodium iodoxybenzoate, justify our conclusion that this drug exercises a definite inhibiting effect on the intracutaneous reaction on local application, particularly during the earlier stages. At the same time these observations also do not favor the conception that the diminishing influence of intravenous injections of this substance on the intracutaneous reaction is dependent on a general toxic effect.

Considering the possibility that oxidative processes might be concerned in the effect of the sodium iodoxybenzoate on the intracutaneous reaction experiments were made to test the influence of hydrocyanic acid. These experiments were limited to the investigation of the local influence of sodium cyanide in the same manner in which the local influence of iodoxybenzoic acid was studied.

Animals Nos. 4, 14, and 19 received on April 4 on the right side of the abdomen 0.1 cc. serum diluted 1 : 5 with a 0.5 per cent solution of sodium cyanide, on the left 0.1 cc. serum diluted 1 : 5 with saline, while a control injection of 0.1 cc. 0.5 per cent sodium cyanide was given in a third place. The results are as follows:

		TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
4	R.....	21.5	24.0	24.5	19.5
	L.....	13.5	19.5	19.5	16.5
	C.....	0.0	0.0	0.0	0.0
14	R.....	17.8	22.5	21.5	22.0
	L.....	11.5	17.0	19.5	20.0
	C.....	0.0	0.0	0.0	0.0
19	R.....	21.5	26.0	26.0	22.5
	L.....	14.5	21.0	21.0	18.0
	C.....	15.0	16.0	+	0.0

The total diameters are:

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
R.....	20.2	24.2	24.0	21.3
L.....	13.2	19.0	20.0	18.2
C.....	5.0	5.3	0.0	0.0

The average diameters of the reactions of the same animals on April 11 and 19 were:

April 11	{ R.....	18.7	19.8	22.5	18.5
	{ L.....	18.7	18.7	20.2	16.3
April 19	{ R.....	16.0	18.7	19.3	17.0
	{ L.....	15.3	19.2	20.2	17.7

In these experiments the most pronounced difference in the reactions was noted after two hours; later on this difference was much less striking.

The second series on April 30 comprises animals Nos. 1, 2, 7, 8, 14, 17, 19. The arrangement of the experiment was the same with this difference that the strength of the NaCN solution was 1 per cent.

		TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
1	{ R.....	23.0	25.0	26.0	25.0
	{ L.....	18.5	20.5	24.5	22.0
	{ C.....	17.0	17.0	17.0	12.5
2	{ R.....	20.5	20.5	22.0	17.0
	{ L.....	15.5	19.5	20.5	16.5
	{ C.....	14.5	14.5	14.5	0.0
7	{ R.....	21.0	22.0	20.5	16.5
	{ L.....	18.0	20.5	19.0	15.0
	{ C.....	20.0	23.5	17.0	11.5
8	{ R.....	26.0	26.0	28.0	23.0
	{ L.....	16.5	21.5	21.5	24.0
	{ C.....	0.0	0.0	0.0	0.0
14	{ R.....	19.0	19.5	18.0	17.5
	{ L.....	11.0	15.5	13.5	13.5
	{ C.....	12.0	13.0	13.0	14.0
17	{ R.....	22.5	25.5	28.5	27.5
	{ L.....	17.5	23.0	26.0	26.5
	{ C.....	18.0	13.0	0.0	0.0
19	{ R.....	22.0	22.0	22.0	18.5
	{ L.....	13.0	15.0	15.0	13.5
	{ C.....	11.5	0.0	0.0	0.0

The total average of the diameters of this series is:

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
R.....	22.0	22.9	23.6	20.6
L.....	15.7	19.4	20.0	18.7
C.....	13.3	11.6	8.8	5.4

The control experiments with the same animals on April 11 and 19 gave these averages:

		TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
April 11	{ R.....	21.5	24.7	26.8	23.1
	{ L.....	21.3	23.6	25.2	21.1
April 19	{ R.....	17.3	20.4	21.0	18.9
	{ L.....	17.1	20.4	21.1	19.1

In this series, as in the preceding one, the difference between the reactions, striking after two hours, became less distinct as the time advanced.

It was suspected that the initial increase of the serum cyanide reactions might be attributable to an addition of the effect of the sodium cyanide as evinced in the controls to the effect of the serum, whereby the alkalinity of the cyanide solution may have played a rôle.

The solution of sodium cyanide employed in the following experiments was prepared with special care. Its strength was one per cent verified by the determination of its CN content. The alkalinity was also determined and corresponded to that of a 0.64 per cent sodium hydroxide solution. The animals Nos. 1, 2, 8, 17, and 19 received May 18 on the right side of the abdomen 0.1 cc. serum diluted 1 : 5 with this sodium cyanide solution, on the left 0.1 cc. serum diluted 1 : 5 with 0.64 per cent sodium hydroxide solution and in a third place 0.1 cc. of the latter.

		TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
1	R.....	24.5	27.5	29.0	27.0
	L.....	14.0	20.0	22.5	21.5
	C.....	10.5	11.0	11.0	0.0
2	R.....	23.0	29.0	29.5	25.0
	L.....	15.0	20.0	23.0	25.5
	C.....	14.0	10.5	10.5	12.0
8	R.....	23.0	25.5	25.5	19.0
	L.....	15.5	20.5	25.5	20.0
	C.....	15.0	13.5	13.5	21.0
17	R.....	22.5	27.5	29.5	26.0
	L.....	16.0	19.0	19.5	18.0
	C.....	0.0	0.0	0.0	0.0
19	R.....	22.5	26.0	30.0	23.0
	L.....	14.5	22.5	26.0	22.0
	C.....	12.5	13.5	13.5	13.0

The averages are,

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
R.....	23.1	27.0	28.7	23.8
L.....	15.0	20.4	23.3	21.4
C.....	10.4	9.7	9.7	9.2

Curve *E* illustrates these findings.

The results of the control experiments with these animals are given on page 29.

The control injections with sodium hydroxide showed little infiltration, but like the reactions with the serum diluted with the NaCN solution, nearly all lead to a slight necrosis.

The differences observed in this series were most striking. In Nos. 2, 8, and 19 this difference was still very pronounced after four hours, while later on the degree of infiltration did not differ as much. In Nos. 1 and 17 a pronounced difference persisted throughout. Similar experiments were made using a 1 per cent solution of hydrocyanic acid in place of the sodium cyanide solution. To these we referred above. The experiments could not be utilized for our purposes here because the addition of the hydrocyanic acid to the serum produced a very fine precipitate.

In the experiment just cited the sodium cyanide exercised a marked influence on the intracutaneous reaction, increasing its intensity particularly in the earlier stages. This observation lends a strong support to the conception that oxidative processes play a rôle in the intracutaneous reaction for Geppert (23) has shown that in the presence of HCN the tissues cannot combine with oxygen. The fact that the sodium cyanide acts in the opposite direction of sodium iodoxybenzoic acid is very suggestive in this connection.

Having established the influence of iodoxybenzoic acid on the intracutaneous reaction it was necessary to see whether a similar influence on the general allergic reaction existed. For this purpose it was our intention to study the influence of this drug on the anaphylactic shock in guinea-pigs. Since we were unable to keep a larger number of these animals we had to abandon this procedure. Dr. Hirschfelder tested the influence of intravenous injection of iodoxybenzoic acid on the effect of intravenous injections of Witte's pepton in the guinea-pig, an effect very much like the anaphylactic shock. No appreciable influence could be detected. In the following experiments we made use of the fall of blood pressure noted in sensitized dogs on reinjection, first described by Biedl and Kraus (24). Three dogs were sensitized with horse serum, all three giving a very good intracutaneous reaction after three weeks. In control animals the intracutaneous injection of our serum did not give any reaction, indeed after two hours it was not possible to recognize the places of injection. One of the animals, weighing 5.6 kg. received then within ten minutes 30 cc. of $\frac{N}{10}$ iodoxybenzoic acid as sodium salt in the femoral vein, followed by an injection of 10 cc. horse serum. The slow injection of sodium iodoxybenzoate in the femoral vein had but a minimal depressing effect on the blood pressure, while a deep and lasting fall set in long before the injection of the horse serum was finished. The second dog of 6.5 kg. received in the femoral vein 10 cc. of a mixture containing 9 cc. serum and 40 cc. $\frac{N}{10}$ sodium iodoxybenzoate. A fraction of a cubic centimeter entered the circulation prematurely producing a slight, but well marked and transitory fall of pressure. With the injection of the mixture the

blood pressure fell rapidly and lastingly. When it had reached an even low level 10 cc. $\frac{N}{10}$ sodium iodoxybenzoate was injected twice without any appreciable effect.

The third dog of 5.5 kg. received on May 18 on each side of the spinal column in shaved places 0.1 cc. serum diluted 1 : 5 with saline. The reactions were intense and agreed very closely in size and degree of infiltration.

The diameters were:

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
R.....	37.5	50.5	47.5	47.5
L.....	37.5	47.0	47.0	46.5

May 26 the dog received in the right side 0.1 cc. serum diluted 1 : 10 with $\frac{N}{10}$ sodium iodoxybenzoate, on the left 0.1 cc. serum diluted 1 : 10 with saline; and in a third place 0.1 cc. $\frac{N}{10}$ sodium iodoxybenzoate as control.

	TWO HOURS	FOUR HOURS	SIX HOURS	TWENTY-FOUR HOURS
R.....	19.0	28.5	42.5	42.5
L.....	23.0	30.0	45.0	45.0
C.....	17.5	17.5	15.0	15.0

The first four hours there was a marked difference between the reactions even more in degree of infiltration than in size, later on the reactions showed practically no difference. All the intracutaneous reactions of our dogs were associated with rather pronounced haemorrhages, leaving a small necrotic area.

On the day of the experiment just reported the dog was less lively than before and coughed. June 6 it coughed considerably, but was still fairly lively and ready to play. On this day it received on either side of the back 0.1 cc. serum diluted 1 : 10 with saline, immediately followed by a very slow injection of 45 cc. $\frac{N}{10}$ sodium iodoxybenzoate in the femoral vein laid bare under cocaine. The result was:

	TWO HOURS	FOUR HOURS	SIX HOURS
R.....	14.0	24.5	15.0
L.....	15.0	16.0	17.0

The result is in accordance with our experiments in rabbits. Two hours after the injections of the serum the animal was rather droopy, with embarrassed respiration. After four hours it was quiet, the respiration somewhat labored, with a good pulse of 136 beats per minute. After six hours the pulse was the same, the respiration labored, 92 per minute. The animal was quiet, somewhat droopy, but easily roused, taking food well. At 10.30 the next morning the dog was found dead, still warm, without rigor mortis. At the autopsy the two upper lobes of the lungs were found solid, of grayish color. The cut surface showed some edema with yellowish white pus exuding. The left lower lobe did not show any lesions, while the right lower lobe was congested, dry and sandy, not containing any air. While it is not unlikely that the diminution of the intracutaneous reaction is chiefly attributable to the effect of the drug, the conditions of this experiment do not permit a definite conclusion.

Our experiments show that iodoxybenzoic acid administered intravenously or locally in rabbits diminishes the intracutaneous reaction. Administered locally in the dog, the same effect is noted and there is an indication that the intravenous administration in the dog has the same effect. At the same time the intravenous administration in the dog does not seem to exercise any influence on the general allergic reaction of this animal.

There seems to exist a certain analogy between the action of iodoxybenzoic acid and the action of larger doses of serum suggesting the production of an anergy. But this analogy is rather superficial. For injections of serum in a sensitized dog, for instance, render subsequent injections inactive, while the iodoxybenzoic acid does not. The mode of action of the iodoxybenzoic acid must be different.

It is self-evident that some differences exist between the general allergic reaction and the intracutaneous reaction. In one case

we have, for instance, a fall of blood pressure, in the other a localized inflammation. It may be possible that the differences between these manifestations are deeper than can be attributable to the effect of the same toxine on a different organ system, an additional reason why substances influencing the intracutaneous reaction may be without effect on the general allergic reaction. Clinically this is of importance, since drugs preventing the eruptions of the skin need not prevent the advent of symptoms comparable to other allergic phenomena and vice versa. In this connection we may cite some observations on rabbits and guinea-pigs sensitized with Vaughan's sensitizing residue obtained from egg white. We owe the material to the kindness of Dr. Vaughan. Animals can be sensitized with this residue, but it is supposed (25) that on reinjections this residue does not give rise to any toxic symptoms, while the unbroken egg white does. Four rabbits sensitized with this residue gave a definite intracutaneous reaction on reinjections with 0.05 to 0.1 cc. of a 10 per cent solution. Some guinea-pigs sensitized with the residue also gave a positive intracutaneous test, while they did not respond to rather large doses given intraperitoneally like 3.0 cc. of a 10 per cent solution. About one-third to one-half of our sensitized guinea-pigs gave a positive intracutaneous reaction with the sensitizing residue. These animals, as the others, gave a systemic reaction with unbroken egg white, with one exception where the intracutaneous reaction was very pronounced, while an intraperitoneal injection of unbroken egg white the next day had no effect. The impossibility of taking proper care of a larger number of guinea-pigs prevented us from pursuing these experiments any further. Such observations would lend strong support to the view that rather far reaching differences may exist between local and general allergic reactions, provided that the sensitizing residue does not contain any unbroken egg white. But Dr. Vaughan notified us "The possibility that in our first residue some unbroken egg white might have been carried over in the residue must be acknowledged." This possibility renders our observations less conclusive, although in view of the quantitative relationships in our experiments it is not excluded that such differ-

ences between local and systemic allergic reactions may perhaps enter in consideration.

The results of our experiments show that the iodoxybenzoic acid has no influence on the general allergic reaction, while it has a definite influence on the local reaction. We are inclined to attribute this effect to a favorable influence on oxidative processes, the more so as cyanide has been shown to have the opposite effect. The local reaction differs from the general particularly in as much as it is an inflammatory reaction of a certain type. Therefore, it is very probable that the influence exercised by sodium iodoxybenzoate and sodium cyanide is directed not so much against the mechanism of the allergic reaction, but against the processes elicited by the product of the allergic reaction. Since this process is here of an inflammatory character we would have to express our results in the following terms: sodium iodoxybenzoate inhibits inflammatory processes while sodium cyanide favors them. Generalizing this expression, we arrive at the hypothesis that any measures favoring oxidative processes in the tissues tend to inhibit inflammatory processes, while measures interfering with oxidative processes tend to favor inflammatory processes.

In closing our paper we may cite one more experiment, prompted by the investigations of Banzhaf and Famulener. A rather emaciated rabbit, weighing 750 grams sensitized to horse serum, received intravenously 12 cc. of a $\frac{N}{10}$ chloral hydrate solution, immediately followed by an intracutaneous injection of serum. The narcosis, established before the injection of chloral hydrate was finished, lasted about one hour. The intensity of the intracutaneous reaction did not show any marked difference when compared with previous reactions. This result prevented further experiments at the time.

CONCLUSIONS

1. The intensity of the intracutaneous reaction of a rabbit sensitized to horse serum is dependent on the amount of serum used for the intracutaneous injection.

2. An intravenous injection of a large dose of serum in a sensitized rabbit diminishes the intensity of the intracutaneous reaction.

3. Iodoxybenzoic acid administered intravenously in rabbits diminishes the intensity of the intracutaneous reaction, while iodbenzoic and benzoic acid in equimolecular concentration do not. Iodoxybenzoic acid administered locally in rabbit and dog inhibits the intracutaneous reaction for several hours, while sodium cyanide, tested in the rabbit, has the opposite effect. Iodoxybenzoic acid administered intravenously in the sensitized dog does not interfere with the fall of blood pressure on reinjection of serum.

4. There is a strong possibility that the iodoxybenzoic acid and the cyanide do not influence the mechanism of the allergic reaction as such, but that their influence is directed against the inflammatory processes due to a product of the allergic reaction.

5. The hypothesis is advanced that measures favoring oxidative processes in the tissues tend to inhibit inflammatory processes, while measures interfering with oxidative processes have the opposite tendency.

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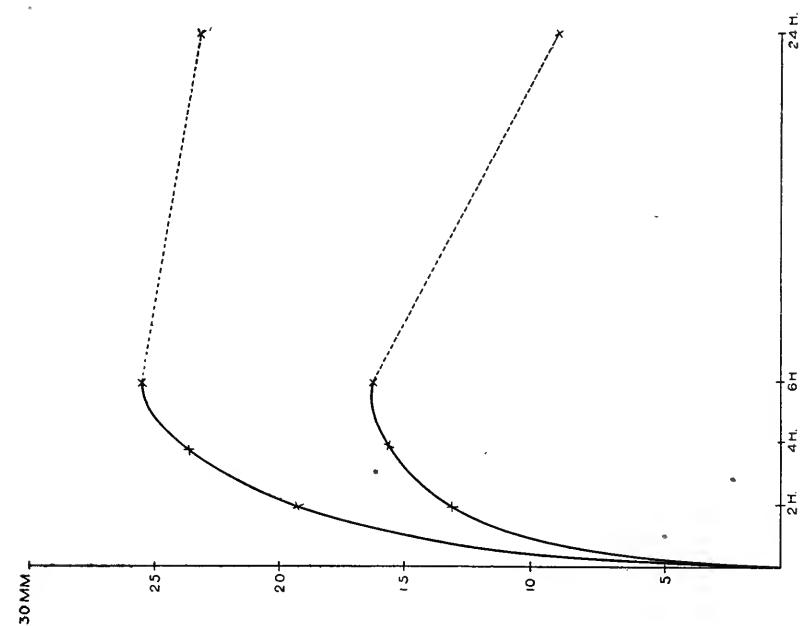
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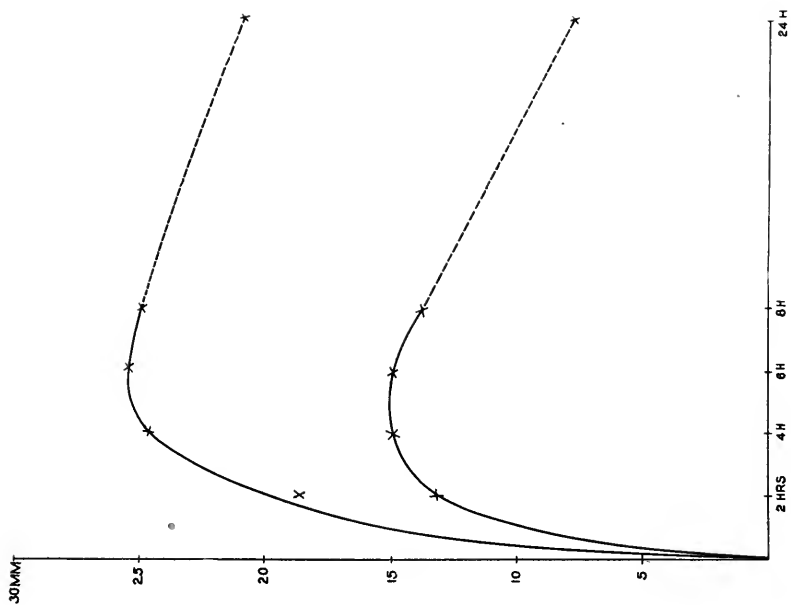
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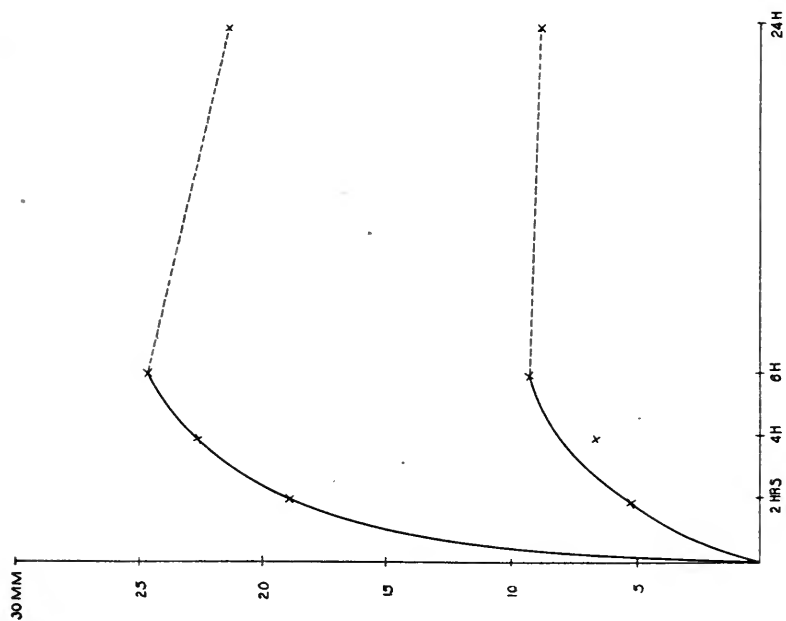
A

Upper curve : Serum diluted 1:10 with saline.
Lower curve : Serum diluted 1:66.6 with saline.



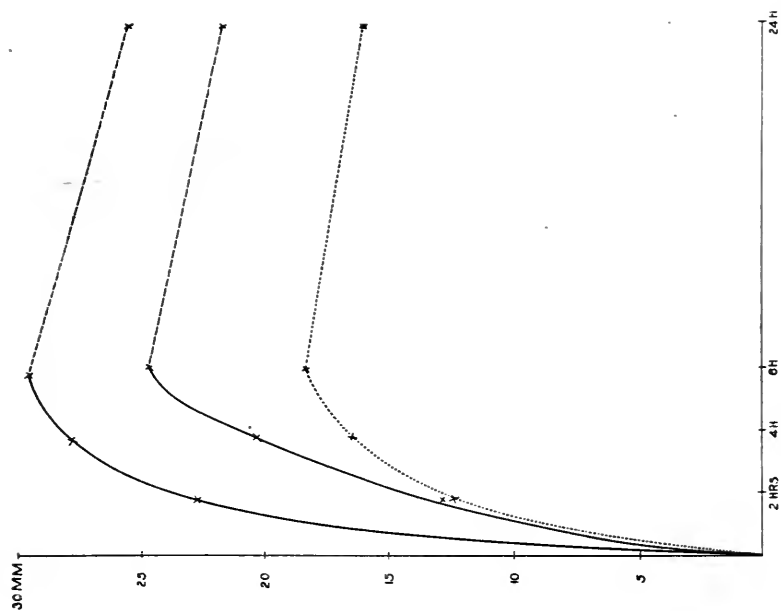
B

INTRACUTANEOUS INJECTION OF 0.1 CC. SERUM DILUTED 1:5 WITH SALINE.
Upper curve : Before the intravenous injection of larger doses of serum.
Lower curve : After the intravenous injection of larger doses of serum.



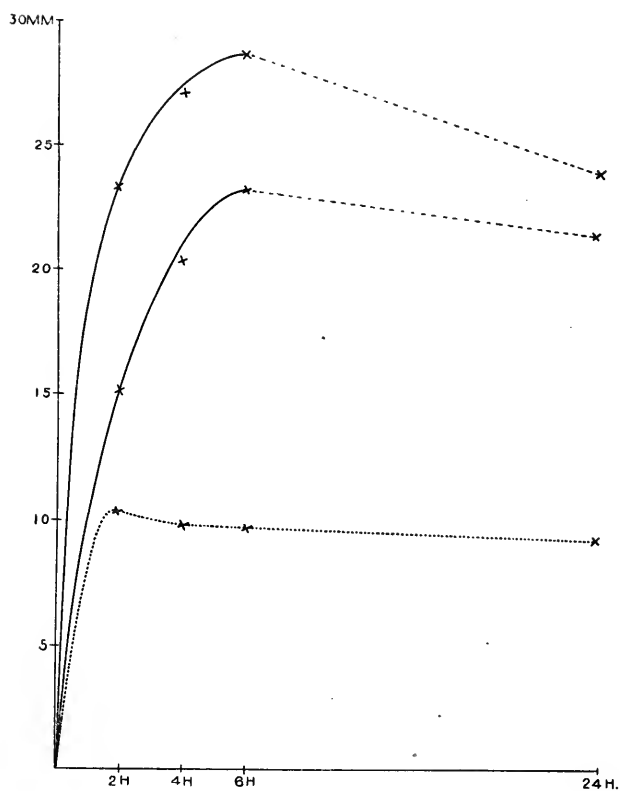
C

INTRACUTANEOUS INJECTION OF 0.1 CC. SERUM DILUTED 1:5 WITH SALINE.
 Upper curve: Before the intravenous injection of sodium iodoxybenzoate.
 Lower curve: After the intravenous injection of sodium iodoxybenzoate.



D

Serum diluted 1:5 with saline.
 Upper curve: Serum diluted 1:5 with saline.
 Lower curve: Serum diluted 1:5 with sodium iodoxybenzoate.
 Dotted curve: Control with sodium iodoxybenzoate.



E

Upper curve: Serum diluted 1:5 with sodium cyanide.

Lower curve: Serum diluted 1:5 with sodium hydroxide.

Dotted curve: Control with sodium hydroxide.

ALCOHOL AND CAFFEIN: A STUDY OF ANTAGONISM AND SYNERGISM

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INTRODUCTION

The objects of the series of experiments are partly practical and partly theoretical.

1. *Practical.* This includes the value of caffein in alcoholic poisoning and the reverse; the proper dosage; methods of administration, etc.

2. *The action of drugs in abnormal physiological states.* Pharmacology has accumulated many data on the actions of drugs on the normal organism. The knowledge of the actions in abnormal states is not so far advanced—although of great theoretical as well as practical significance. Sometimes the actions are the same, resulting in a simple algebraic summation; but it is known even now that often the actions differ quantitatively and qualitatively from such algebraic summations. Before this can be understood, empirical data must be accumulated. This laboratory has for some time been engaged on this study along various lines. One of these is the antagonism and synergism of drugs, for drugs form one of the readiest means of inducing definite, analyzable alterations in functional states. It may be hoped, and reasonably expected, that knowledge so gained may eventually be applicable to those natural alterations in functional states which constitute disease. In the present investigation, caffein and alcohol were chosen, because they are supposed to have opposite actions, while, from their chemical nature, their point of attack on the cell must be different.

Methods: Cats were used exclusively. To observe the effect of caffeine on different degrees of alcoholic intoxication, alcohol was administered in increasing quantities up to the lethal point; caffeine was also administered in increasing quantities. This constitutes three series of experiments: alcohol alone (Part I); caffeine alone (Part II); and the two combined (Part III); the former two series serving as "controls" for the latter.

Ninety-five per cent alcohol was employed diluted to 25 per cent and was administered by stomach tube; the data will be stated as cubic centimeters of the 95 per cent alcohol per kg. of animal. In one series the alcohol was injected into the peritoneal cavity.

The caffeine was administered as a 1 per cent aqueous solution of the free base. The doses always mean absolute caffeine. In some of the experiments the solution was given by the stomach-tube; in the alcohol experiments the two drugs were thus introduced simultaneously. In an equal number of experiments, the caffeine solution was administered hypodermically, after the symptoms of alcoholic intoxication had developed—usually within fifteen to thirty minutes. Although the number of experiments in each series was too limited to permit positive conclusions concerning the differences between the gastric and hypodermic administration, the variations as observed did not seem of practical importance and the two methods will not be separated in the reports.

Animals in alcohol narcosis, when not in actual coma, react to hypodermic injections by a temporary improvement of the narcosis. This reaction does not seem to be due to the caffeine, but to the reflex stimulation from the local irritation of the injection, for similar improvement follows on the injection of equal quantities of simple saline solution or of water.

In presenting the results, I shall first discuss the effects of alcohol and of caffeine, when given separately, and then the effects when both drugs are combined. The observations were confined mainly to the following functions: Psychic, motor, heart-rate (per ten seconds), respiratory rate (per fifteen seconds), temperature (Centigrade) and mortality.

Under the term "psychic" will be presented the general behavior of the animals, following the classification of Sollmann and Hatcher (1) for the narcotic effects:

1. Drowsiness.

2. Light natural sleep: the animal slumbers in a natural position, and is easily and completely roused, as by a heavy step on the floor.

3. Deep natural sleep: the animal lies in a natural position, soundly asleep. It is roused only by rather severe stimuli, and then incompletely, appearing stupid, and dozing again very quickly. Forced to its feet, it is very clumsy and walks with a stagger. The reflexes are nearly normal.

4. Light coma: the animal lies extended on its side or belly, relaxed, but with some muscular tone remaining. It may make some spontaneous movements, but is generally unconscious. It responds rather weakly to pain. The reflexes are generally depressed.

5. Deep coma: the animal is completely relaxed and anesthetic. The reflexes disappear, the corneal reflex persisting until rather late.

The experiments in which the animals vomited are not included in the data unless otherwise stated. Vomiting was very frequent with the larger quantities of alcohol; and to avoid this alcohol, 7.5 cc. per kg. was administered intraperitoneally in one series, alone and combined with caffeine; but this was found to be uniformly fatal.

PART I. THE EFFECTS OF ALCOHOL

Psychic or narcotic effects

The first effect of alcohol is often restlessness or slight excitement, lasting fifteen to thirty minutes. This occurred oftener with the smaller quantities, the larger doses resulting in an earlier narcotic and motor depression. With 4 cc. per kg. the maximum narcotic effect was usually reached within an hour; with 7.5 and 10 cc. considerably earlier; with 7.5 cc. intraperitoneally usually within ten minutes.

Alcohol—0.5 cc. per kg. One experiment. No effect was observed.

2.0 cc. per kg. Four experiments. This was the minimum effective dose and produced drowsiness or light sleep for one to two hours.

4.0 cc. per kg. Twelve experiments. Five animals passed into light to deep sleep; six were in light coma for two to three hours; one in deep coma; all normal in twenty-four hours.

7.5 cc. per kg. Five experiments. Three animals were in deep coma through five hours; two of these were normal in twenty-four hours; one in light sleep on the second day, dying on the fourth day. Of the other two, one was in light coma and one in deep sleep during the day with recovery in twenty-four hours.

10.0 cc. per kg. Five experiments. All were in deep coma during five to six hours. In twenty-four hours there was one complete recovery; one in deep sleep with recovery on the third day but with death on the fourth day; one made no recovery from coma, dying during the third night. Two animals vomited, and of these one died in twenty-four hours, the other on the third day after partial recovery. Other animals vomited and recovered.

7.5 cc. per kg. intraperitoneally. Three experiments. One animal was in deep coma within five minutes and a second within five to ten minutes; the third became sleepy promptly, but coma was delayed several hours.

Conclusion. Alcohol has a narcotic effect on cats, increasing quantitatively with the dose; 2 cc. per kg. is about the minimum effective dose, producing drowsiness or light sleep. With 4 cc., there is deep sleep or light coma. Larger doses, 7.5 to 10 cc., cause deep coma, from which there may be complete recovery, or recovery may be temporary with death later, or death may ensue without improvement from the coma.

Motor Effects. The motor depression seemed to come on earlier and to be of longer duration than the mental depression. Not infrequently an animal would be seen lying down with head erect apparently paying attention to its surroundings, but practically unable to stand when disturbed; again animals in sleep from which they could be readily aroused would be unable to rise to their feet. This was especially noticeable in a series of three experiments in which two animals did not pass beyond light sleep and one beyond deep sleep, but all were practically unable to stand for three hours and still displayed considerable incoördination in five hours. Complete motor recovery was thought to have been reached when an animal could jump from a table without losing its balance.

The number of experiments with each dose is as stated under the "Psychic Effects."

Alcohol—0.5 cc. per kg. No effect was observed.

2.0 cc. per kg. Moderate incoördination for three or four hours, with complete incoördination for one hour in one experiment; reflexes slightly depressed.

4.0 cc. per kg. Incoördination practically complete within one hour, with improvement in two or three hours and nearly normal in six hours; in two animals the incoördination was much less and in one recovery was delayed; reflexes greatly depressed.

7.5 cc. per kg. Incoördination complete within fifteen or thirty minutes lasting through six hours; animals still weak on the second day. Two animals showed occasional rythmic asymmetrical movements of the legs; reflexes abolished.

10.0 cc. per kg. Incoördination complete through six hours. One animal was about normal in twenty-four hours, and another in forty-eight hours; one showed no recovery, death occurring on the second night. Two animals vomited but were as depressed as the others. Rythmic flexion and extension of the legs was seen in one animal which recovered and in one which died. The reflexes were abolished.

7.5 cc. per kg. intraperitoneally. Two animals were completely ataxic in two or three minutes; a third was able to walk fairly well for twenty minutes, becoming ataxic later; the reflexes were abolished early.

Conclusion. Alcohol causes a motor depression in cats, increasing with the dose. Incoördination or ataxia is complete with 4 cc., sometimes with 2 cc. This depression comes on more rapidly and recovery is delayed more than the psychic depression, otherwise the two run fairly parallel.

Fatality (see tables II and III, page 285). The lethal quantity of alcohol per os for cats lies close to 10 cc. per kg.; although late death may occur exceptionally with smaller doses. 7.5 cc. per kg. intraperitoneally is more toxic than 10.0 cc. per os. Coma lasting into the second day was always fatal, although apparent recovery may take place before death. This observation agrees with a statement of Cushny (2) and Kunkel (6) that persons are said rarely or never to recover if unconsciousness lasts longer than ten to twelve hours after the drinking bout.

Alcohol—0.5 to 2.0 cc. per kg. None.

4.0 cc. per kg. One death in twelve experiments. Death occurred the next morning after practically complete recovery; in twenty-four hours the laboratory assistant said this cat was in the same condition as the others but suddenly went into a fatal convulsion. This death is so exceptional, that it might be considered an accidental fatality.

7.5 cc. per kg. One death in five experiments on the fourth day after partial recovery.

10.0 cc. per kg. Four deaths in five experiments. Two deaths occurred within twenty-four hours with no recovery from coma (one of these animals vomited). The third death occurred during the second night after partial recovery from the narcosis but almost no motor improvement. The fourth cat was still quite depressed on the second day but seemed normal on the third day, excepting a 4 per cent loss in weight; but it was found dead on the fifth day. The one recovery animal was normal within twenty hours, although somewhat weak.

7.5 cc. per kg. intraperitoneally. In three experiments death ensued respectively in seventy minutes; two to three hours, and over night.

Effects on the heart rate. The average heart rate in eighty-six cats was 33 per ten seconds with a maximum of about 40, and a minimum of 20. This average is probably somewhat higher than the normal rate, as the cats were usually somewhat restless when first examined, although the rate was not taken until they apparently became quiet. They usually became accustomed to handling. To count the rate, a stethoscope was used, as this was found to be much more exact than palpation; with a rapid heart palpation was quite unsatisfactory.

Alcohol—0.5 cc. per kg. In one experiment no effect was observed.

2.0 cc. per kg. The average of the counts shows a decrease in rate of two to six beats per ten seconds; an increase (of seven per ten seconds) occurred in one experiment only, at the third hour. The slight degree of narcosis could readily account for the decrease in rate.

4.0 cc. per kg. The averages show a very slight increase (2 per ten seconds) in rate at the third hour with return to normal at the fourth hour. Eight experiments gave an increase, three no change, two a decrease. The maximal increase was from 30 to 37 per ten seconds; the maximal decrease from 33 to 25, at the first hour (AA1).

7.5 cc. per kg. The averages show a decrease of 2 to 3 beats per ten seconds after the first hour; on the second day an increase from 34 to 38 per ten seconds, of forty-eight hours duration. The maximal increase was from 38 to 41 at the fifth hour (IA5) and this is the only recorded increase; the maximal decrease was from 33 to 27 at the fifth hour (FF2) and this animal gave the maximal increase (to 46) on the second day.

10.0 cc. per kg. There was an average decrease of 3 to 5 beats per ten seconds, persisting over five hours; with a maximum decrease from 36 to 21 at the second and third hour (IIA8); the maximum increase was from 31 to 37 at the second hour (IIA1) and but three increased rates were recorded. In twenty-four hours one rate was decreased nearly 50 per cent (from 36 to 20); one was unchanged; one was increased from 28 to 47 per ten seconds.

7.5 cc. per kg. intraperitoneally. In two animals with early coma and death, the rate was decreased from 25 to 50 per cent, and was irregular; in the third animal (FF23) the rate was decreased from 30 to 25 in three hours and then was increased to 36 per ten seconds.

Conclusion. With small quantities of alcohol (2 to 4 cc. per kg.) the heart rate may be unchanged or slightly increased or decreased; with quantities (7.5 to 10 cc. per kg.) leading to deep coma for several hours or longer, there is usually a decrease in rate of 2 to 5 per ten seconds during the first six hours; although there exceptionally may be a much more marked decrease. With the larger quantities there is a marked variation in rate in twenty-four hours, ranging between a decrease or increase of 50 per cent or no change.

Temperature. The effect is shown in the accompanying curve (fig. I, page 274). The rate and extent of the fall of temperature, and the time required for recovery, increase with the dose of alcohol. The average fall for successive doses may be stated as 0.75° Centigrade, in two hours for 2 cc.; 1.9° in three hours for 4 cc.; 2.8° in two hours for 7.5 cc.; and 3.8° in two to five hours for 10 cc.

Alcohol 2.0 cc. per kg. The average loss was 0.75° in two hours with recovery in about four hours; the maximum loss was 1.2° and the minimum 0.2° .

4.0 cc. per kg. The temperature falls rapidly, eight experiments showing an average loss of 1.5° in one hour. The fall continues to the third hour, when ten experiments show an average fall of 1.9° . Recovery begins usually between the third and fifth hours, but may be earlier or somewhat delayed. The normal may be reached in five to six hours; and all the temperatures were normal in twenty-four hours. The greatest fall recorded was 3.1° at the third hour (EE11). The smallest fall (0.9° at the end of the first hour) was shown by an animal (EE1) which showed a subsequent rise of 0.8° at the end of six hours.

7.5 cc. per kg. The loss was both faster and greater than with 4.0 cc. The average fall at one hour was 2.1° , and at two hours 2.8° . Recovery started before the fifth hour in two of five experiments. The greatest fall recorded was of 4.0° at five hours (IA1); the smallest of 2.4°

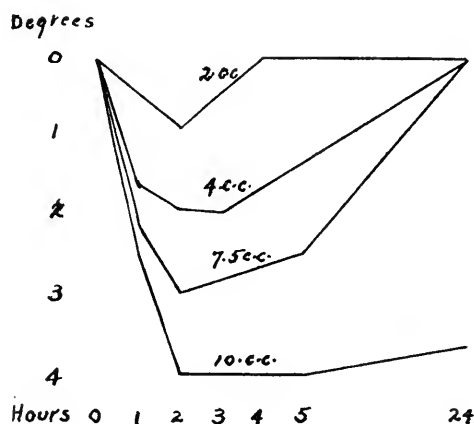


FIG. 1. ALCOHOL ON TEMPERATURE
(Alcohol cc. per kg.)

at the second hour (FF2). (Temperatures were not taken in all experiments between the second and fifth hours, or greater variations might have been recorded.) At twenty-four hours two temperatures were normal, two subnormal (-1.7° ; -0.9°) and two above normal (1.2° ; 0.9°).

10.0 cc. per kg. The fall continued to increase in rate and degree. The average was 2.4° in one hour, 3.8° in two and five hours. In two of three experiments the temperature had somewhat recovered at five hours. The greatest fall was 7.5° at the fifth hour, the smallest 3.6° . In twenty-four hours all were still subnormal: 0.7° , 3.1° , 8.0° ; the former

two animals recovered, but the last died. In experiments with death on the third and fourth days, the temperature was normal on the second day.

7.5 cc. per kg. intraperitoneally. In one experiment the fall was 4.3° in five hours; the animal was then placed in a hot room and the temperature was normal in three hours. The animal died during the night.

Respiration. Small quantities of alcohol (2 cc.) cause a slight (15 per cent) increase in respiration, lasting for two or three hours; with larger quantities (4 cc.), which cause narcosis varying from deep sleep to deep coma, the rate is about doubled during the first hour with gradual return to normal in three to five hours; with still larger quantities, 7.5 to 10.0 cc., the rate is usually increased for several hours, but it may be unchanged.

Alcohol—2 cc. per kg. Two animals showed an increase, one no change, and one a decrease. The average of the four experiments shows a slight increase (from 6 to 7 per quarter minute) for two hours, with return to normal in the third hour.

4 cc. per kg. Seven animals all showed a marked increase. In six of these, the average rate rose from 6 to 12 (per quarter minute) at the second hour. In the seventh, the increase was from 7 to 30. In three hours the rate was again practically normal.

7.5 cc. per kg. Three animals gave a 50 per cent increase (from 8 to 12 per fifteen seconds) for practically three hours, with return to normal in five hours. Only one experiment gave a slight decrease after the second hour.

10 cc. per kg. Two experiments. In one the rate was, 8, 12, 15, 9 at 1, 3, 5, 24 hours, in the other the rate was, 3 to 4, 3 to 4, 3 to 4, 2 to 3, at 1, 3, 5, 24 hours. This gives an average slightly above normal during the day but below normal in twenty-four hours.

7.5 cc. per kg. intraperitoneally. In one experiment the respiration was greatly slowed and gasping, 8 per minute; in a second it became imperceptible in ten minutes and then returned to normal (animal was not seen at death); in the third the rate was increased at the second hour and then returned to normal.

PART II. SUMMARY OF THE EFFECTS OF CAFFEIN

The caffein was administered in 5, 15, 30, 60, 120 and 200 mg. doses (per kg.). The results with hypodermic and gastric admin-

istration offered no practical differences and will not be distinguished in this summary. To avoid the interference of vomiting, the 120 and 200 mg. doses were given hypodermically only.

Psychic effects

Non-fatal doses of caffeine cause only wakefulness and increased irritability. In fatal experiments there is excitement, varying in degree from slight restlessness to intense excitement; followed in either case by convulsions.

Caffein—5 to 30 mg. per kg. There were four experiments with 5 mg.; four with 15 mg.; three with 30 mg. During six hours all the animals remained wakeful; one 15 mg. animal was seen to doze occasionally; one animal was restless before receiving 15 mg. per kg., which did not increase the restlessness; they were thought to be a little more irritable than normally; no other effects could be observed.

60 mg. per kg. Eight experiments. Four animals were in poor condition from distemper; three of them were drowsy most of the time and one was somewhat restless, walking about the cage for two hours and then becoming quieter. Four animals were in good condition; two of these were restless and quarrelsome for two or three hours and were then quiet; one was quiet at all times; one restless for two hours and was found in rigor mortis at four hours.

120 mg. per kg. Seven experiments. Four animals had distemper and were in poor condition; of these, one died in one hour, in convulsions, after a period of restlessness. Of the three healthy animals one died in thirty-five minutes (death not seen). All the others were restless for two or three hours and rather quarrelsome, and then were quieter but wakeful. There was no excitation such as is caused by morphin. Salivation was common.

200 mg. per kg. One experiment intraperitoneally: The animal shortly became excited as with morphin, rushed about the cage and ran into the sides of the cage, eyes wide open and "glaring," salivation, continued crying; death from one convulsion in twenty minutes.

One experiment by stomach. The animal became slightly excited, with death in fifteen or twenty minutes from one convulsion.

One experiment by stomach with 0.5 cc. per kg. alcohol. The animal was wide awake but remained quiet; death in forty minutes from one convulsion when picked up for examination.

Two experiments, one hypodermatically and one intraperitoneally in which the animals were in deep coma from alcohol 10 cc. per kg. and had vomited. One died in just twenty-four hours and the other in two hours, neither improving from deep coma.

One experiment intraperitoneally in which animal was somewhat depressed by alcohol (had vomited). This animal became restless; death in three hours from convulsions.

Motor Effects. Caffein increases the reflex excitability. This irritability was slight with small doses and increased roughly with the dose; in lethal quantities death ensued during the convulsions. There may be marked activity before the onset of convulsions, but this occurred in but one of four deaths.

With 5, 15 and 30 mg. doses the reflexes were more active, very slightly so with the smaller doses; no other abnormalities observed.

With 60 and 120 mg. doses the reflexes were markedly increased during the day, but the animals were handled carefully to avoid causing convulsions.

The one death with 60 mg. was not seen. One of the two deaths with 120 mg. was seen and presented the following phenomena:

Experiment P1,—Caffein 120 mg.: 9:30 a.m. Cat received 120 mg. per kg. hypodermatically.

Thirty minutes—Has been somewhat restless but otherwise normal.

Sixty minutes—Restless, but is now lying down, has just stood on all fours and is trembling violently; back arched and head thrown backward; turned a "forward sommersault;" legs moving rapidly and incoördinately; hair erect; salivation. General incoördinate convulsions, very fine, lasting two or three minutes; is now lying on its side breathing deeply and rapidly, preventing heart observations.

Eighty minutes. Animal went into a less severe convulsion of 2 minutes duration. Shortly a third convulsion; respiration ceased, heart beating regularly for one minute; then one respiratory gasp and heart became irregular, continuing to beat for sixty seconds.

With 200 mg. per kg. One of three animals became very active as was described above under "psychic effects;" two others went into convulsions without previous marked motor excitation.

Heart rate. With small doses caffein may cause a slightly increased heart rate; with increasing doses the rate increases up

to a maximum of 50 or more per ten seconds. This maximum rate was not attained with 120 mg. in two normal animals, but was attained by two of four animals with 60 mg. and with 120 mg. in three of three animals, all of which were in poor condition from distemper. It would seem then that reduced vitality renders the heart more susceptible to large doses of caffeine. With large doses—30 mg. and up—the heart rate is increased by roughness in handling. No irregularities were observed with any of these quantities except with the onset of convulsions.

Caffein—5 mg. per kg. Four experiments. There was practically no effect; two experiments showed no change in rate; one showed a slight increase (maximum from 21 to 26 per ten seconds); one showed a slight decrease (maximum from 32 to 28).

15 mg. per kg. Four experiments. All showed a slight decrease in rate in one hour (an average from 31 to 29), and then return to normal.

30 mg. per kg. Three experiments. During the first hour the rate was unchanged, and then all gave an increase reaching a maximum (from 26 to 32) at the second hour, remaining at that rate during seven hours and returning to normal in twenty-four hours. Rough handling caused an increased rate somewhat greater than in the normal animal.

60 mg. per kg. Four experiments on normal animals. The averages show an increase in rate (from 25 to 27 or 28 per ten seconds) in one hour and persisting for five hours. Three were normal in twenty-four hours and one was not normal until forty-eight hours. The maximum increase was from 22 to 35; the maximum decrease from 32 to 27 at the second hour, with death in three hours.

Four experiments on animals in poor condition from distemper: The average initial rate was 39 per ten seconds. In two animals the rate was increased to about 50 in one hour; one of these remained at this point during seven hours and became normal in twenty-four hours; the second gradually returned to normal in seven hours. In the other two animals the increase was less marked. In either series, any roughness in handling caused a markedly increased heart rate, much more than is seen in animals that have not received caffeine.

120 mg. per kg. Three experiments with animals in poor condition gave about the same results as with 60 mg. The rate increased to about 50 per ten seconds and this rate was maintained for some hours; the normal not being restored till the second day in one animal, the third day in the others.

Two experiments with normal animals: In these the increase was somewhat less, with return to normal on the second day. Again roughness in handling greatly quickened the rate. The 120 mg. dose caused a more marked and more persistent increase in rate than the 60 mg. dose. There were no irregularities observed except with the onset of convulsions.

200 mg. per kg. The animals were not examined lest convulsions be brought on.

Temperature (see table I, page 279). All quantities gave an increase in temperature usually reaching its maximum in four hours. The maximal temperature, with doses between 15 and 120 mg. per kg., was from 0.8° to 2.0° above the normal, the average maximal rise being about 1.5° . Recovery of the temperature started before six hours and was complete by twenty-four hours.

TABLE I
Caffein on temperature. Average rise in degrees Centigrade

DOSE MG. PER KG.	NUMBER OF EXPERIMENTS	HOURS				MAXIMUM	MINIMUM
		2	4	6	24		
5	4	0.5	0.6	0.5	0	0.9	0.2
15	4	0.7	1.2	0.9	0	1.5	0.9
30	3	1.0	1.1	1.0	0	1.9	0.4
60 well	3	0.7	1.5	0.8	0	2.0	1.0
60 sick	4	0.95	1.1	0.7	0	1.5	1.0
120 well	2	0.9	1.6	1.5	0	1.9	1.3
120 sick	3	1.1	0.95	0.8	0	1.6	0.8

The accompanying table (I) shows that with 60 and 120 mg. the rise is but slightly greater than with 15 mg. per kg., but is maintained somewhat longer with 120 mg. With 60 and 120 mg. the rise was somewhat greater with well than with distemper animals, although the original temperature was practically the same.

From a survey of the literature, it would appear that cats are especially susceptible to the hyperpyretic action of caffein. Thus, Cushny (2) states that caffein raises the temperature by 0.5 to 1° , but only when almost poisonous doses are reached. Binz (8) found a rise of 0.6° with moderate, non-convulsive but

toxic doses, and a rise of 1 to 1.5° with doses which caused muscular rigidity. Our cats showed a rise of 0.6° with 5 mg. per kg., a dose far removed from the toxic, (which would correspond to 5 grains for a 130 pound man); they also showed an average rise of over one degree with 15 mg., which do not cause muscular rigidity.

Rate of respiration. With quantities up to 15 mg. per kg., caffeine had no effect on the respiratory rate; with larger quantities the rate was somewhat increased for two to three hours, with an average maximum increase of about 30 per cent with 120 mg. per kg.

The number of experiments with the different doses has been given above. (Heart.)

5 mg. per kg. The respiration was practically not affected; in no animal did the rate vary more than one per quarter minute.

15 mg. per kg. The average rate was unchanged; in two experiments there was a slight increase and in one a similar decrease.

30 mg. per kg. The average rate was unchanged.

60 mg. per kg. In four experiments on sick animals there was a slight average increase (2 per minute) during two hours. In two normal animals on a very hot day, the rate was somewhat increased in one and decreased in the other (from 12 to 15 and from 9 to 7 per fifteen seconds).

120 mg. per kg. Three distemper and one normal animal all gave an average increase from 5 to 7, 6 and 6 per fifteen seconds, in one, two and three hours, with a normal rate in four hours; the rate in the normal animal was slightly greater. In one animal on a very hot day the rate was increased greatly, from 12 to 15 per fifteen seconds, to 25 to 30 during three hours with return to normal in six hours.

Fatality (see tables II and III page 285). Caffeine 5-30 mg. per kg.: No fatality in eleven experiments; 60 mg. per kg.: one fatality in eight experiments; 120 mg. per kg.: two fatalities in seven experiments; 200 mg. per kg.: three fatalities in three experiments; and also three of three animals that had received non-fatal doses of alcohol.

Fatal dose. No attempt was made to determine the "just lethal" quantity of caffeine; the one death in eight with 60 mg.

and two in seven with 120 mg. shows that it must vary within fairly wide limits. The average lethal point probably lies a little above 120 mg. per kg. In view of the increasing heart rate and cardiac irregularities (to be discussed later), the extreme cardiac dilatation, and the arterial color of the blood post mortem, it would seem that cardiac failure was the cause of death in caffein poisoning. Asphyxia cannot be the cause of this cardiac dilatation as the blood would be venous.

Salant and Rieger (3) state that 0.15 to 0.155 grams per kg. caffein administered subcutaneously to cats produced symptoms in ten to fifteen minutes and death in a little over an hour.

Connection with convulsions. Of four deaths from caffein which were seen all were during convulsions, and from the appearance of the cage two others also died in convulsions. With one exception the convulsions were apparently spontaneous, in the one exception—200 mg.—the animal went into convulsions when picked up for examination.

The convulsions were always fatal. The three 200 mg. animals each had but one attack; the single 120 mg. death had several attacks.

Influence of distemper on the fatality. The animals receiving the 60 and 120 mg. doses were about equally divided between good and poor condition, yet two of three fatalities were among those in good condition. Although the experiments were somewhat limited in number, it might be inferred that a somewhat low vitality does not render the animal hypersusceptible to fatal doses of caffein.

Four caffein animals (three with 200 mg. and one with 120 mg.) were autopsied immediately after death; in all these the heart was dilated to the capacity of the pericardial sac; all chambers were dilated; the lungs were normal in color and the blood bright red, although in one experiment the respiration had ceased before the heart. In a second experiment the heart stopped before the respiration.

In fatalities from caffein alone and combined with alcohol cardiac rigor may ensue very early, especially in the left ventricle which was frequently observed to be contracted to obliteration of its

cavity, shortly after death. The right ventricle and auricles were never seen contracted.

PART III. ALCOHOL AND CAFFEIN COMBINED

Psychic and motor effects

Alcohol alone being a psychic and motor depressant, and caffein alone a stimulant, one would expect their combination to result in typical antagonism. In fact, however, this is very limited, and restricted to relatively small doses of alcohol (to 4 cc.), and of caffein (to 30 mg.). With larger doses of alcohol, small doses of caffein are ineffective, and larger doses (above 30 mg.) are synergistic.

Small quantities of alcohol (2.0 cc.) appear not to be affected by small doses of caffein, up to 10 mg. but the symptoms are not sufficiently definite to secure conclusions. On the contrary, small doses of alcohol, even 2 cc., increase the toxicity of large doses of caffein, 60 to 120 mg.

The depression of large doses of alcohol (10.0 cc.) is increased by caffein in small or large doses (from 5 to 60 mg.). This is also true of alcohol 7.5 cc., except that depressant symptoms are sometimes temporarily obscured by excitement.

With moderate quantities of alcohol (4.0 cc.) caffein 2 to 15 mg. usually lessens the narcosis and hastens recovery, (but occasionally it may increase the initial narcosis). The larger of these doses are the more effective: 30 mg. also hasten recovery but do not effect the initial narcosis; 60 mg., hypodermically, improve the symptoms, but add to the toxicity. The same dose by stomach greatly increases the narcosis and adds to the toxicity.

The effect of caffein on the motor incoördination agrees essentially with the psychic effect; when the narcosis was improved the incoördination was earlier recovered from, and vice versa. With the larger doses of caffein, causing excitation, the motor depression was largely removed although the animals' movements were not purposeful, the animals moving rapidly to and fro in the cages. The reflexes remain depressed in the excited state, there being usually no pain sensation present.

The rhythmic involuntary partial flexion and extension of the legs and flapping of the tail, mentioned in the alcohol series, was observed a little more frequently in this series, in four of ten experiments with 7.5 and 10.0 cc. of alcohol alone and thirteen of twenty-three experiments with the alcohol plus various doses of caffein.

Alcohol—2.0 cc. per kg.

Caffein—2, 5, 10 mg. per kg. Four experiments in all. Caffein had no influence on the drowsiness or light sleep of the alcohol.

60 mg. per kg. Three experiments. There was no narcosis in these animals nor in one control; however, other controls were slightly narcotized by 2 cc. Caffein 60 mg., therefore, probably removes the narcosis of small quantities of alcohol.

120 mg. per kg. Two experiments. One animal was greatly excited as with morphin until death ensued in two hours; the second went into convulsions in thirty minutes without previous excitation.

Alcohol—4.0 cc. per kg.

Caffein—2 mg. per kg. Two experiments. There seemed to be slight improvement.

5 mg. per kg. Seven experiments in two series. In series A, four experiments, the narcosis was somewhat less and recovery earlier than with two controls; in series B, three experiments, the narcosis was deeper for two hours than that of the two controls, but recovery was earlier.

15 mg. per kg. Two experiments. The narcosis was replaced by excitation.

30 mg. per kg. Three experiments. The initial narcosis was not lessened and in one animal it was even increased and death ensued in seventy minutes. The two survivals recovered before the controls, but were excited.

60 mg. per kg. Four experiments. In one experiment the narcosis was greatly deepened with death in forty minutes; in three experiments the animals became excited and died within 2 hours. One animal died in convulsions.

Alcohol—7.5 cc. per kg.

Caffein—5 mg. per kg. Three experiments. There was no effect on the narcosis in two; in the third excitation replaced the narcosis. Recovery was not influenced.

15 mg. per kg. Four experiments. There was no improvement and possibly the narcosis was increased.

30 mg. per kg. Two experiments. The narcosis was increased.

60 mg. per kg. Two experiments. The narcosis was probably increased.

Alcohol—10.0 cc. per kg.

5, 15, 30 and 60 mg. doses of caffeine. Ten experiments in all, the narcosis was deepened. With one 10 mg. dose, there was no effect.

Alcohol 7.5 cc. per kg. intraperitoneally. Six experiments: Caffein from 2 to 30 mg. had no effect on the narcosis.

Binz (4) states that the alcohol narcosis is relieved by caffeine. He cites but one experiment: Two puppies received about 10 cc. per kg. of alcohol hypodermically in divided doses; after complete narcosis one received caffeine, about 50 mg., subcutaneously and the animal became excited in three minutes; both recovered completely.

In this series such large doses were exceedingly dangerous; but not infrequently in these experiments animals which had received smaller doses of alcohol became similarly excited after caffeine; cats and dogs may react differently.

The majority of the texts in pharmacology or toxicology Binz, Cushny, Heinz, Kionka, Kobert, Lewin, Sollmann and Von Jaksch recommend caffeine or coffee infusion in alcohol poisoning. Our results show that the recommendation is justified for the lighter grades of alcoholic intoxication, but as Kunkel (6) correctly states, not much is to be expected from caffeine in severe alcoholic intoxication, indeed, it would then be deleterious.

In our class work, we often have seen the remarkable reviv-ant effects of hypodermic injections of caffeine (20 mg.) on rabbits in alcohol narcosis (6 cc. per kg.). The improvement is immediate and striking, perhaps more so than in cats, but as has been pointed out, the improvement is largely reflex, and this probably also applies to the much quoted experiment of Binz.

Fatality. The fatality rate of alcohol and caffeine alone and combined is presented in the following tables (II and III). With alcohol 4.0 cc., the one fatality recovered from the acute intoxi-

TABLE II
Fatality, alcohol and caffein; alone and combined

ALCOHOL	CAFFEIN	NUMBER OF EXPERIMENTS	DEATHS
<i>c.c. per Kg,</i>	<i>mg. per Kg,</i>		
4		12	1
7.5		5	1
10		5	4
7.5		3	3
Intra per			
	60	8	1
	120	7	2
	200	3	3
2	60	3	0
2	120	2	2
4	30	3	2
4	60	4	4
7.5	30	2	1
7.5	60	2	2
10	5-15	6	6
10	30	2	2
10	60	2	2
7.5	2-30	6	6
Intra per			

TABLE III
Percentage fatality, alcohol and caffein alone and combined

ALCOHOL	CUBIC CENTIMETERS					INTRAPERI- TONEALLY 7.5
	0	2	4	7.5	10	
<i>caffeine mg.</i>						
0	0	0	8.3	20	80	100
5-15	0	0	0	0	100	100
			(8.3)	(20)	(80)	
30	0		66.7	50	100	100
			(8.3)	(20)	(80)	
60	14.3	0	100	100	100	
		(14.3)	(14.3)	(34.3)	(94.3)	
120	28.6	100				
		(28.6)				
200	100					

Alcohol from left to right in cubic centimeters per kilogram; caffein downward in milligram per kilogram. Figures represent the percentage fatality; figures in parenthesis equal the theoretical fatality, namely the sum of the percentage fatality of caffein and alcohol alone; for instance: The percentage fatality of 7.5 cc. alcohol, when given alone is 20; the percentage fatality of 60 mg. of caffein is 14.3. The sum of these (20 plus 14.3 = 34.3) would be the theoretical fatality.

cation but died after struggling on the following morning. With the two combined the fatality begins with alcohol 4.0 cc., caffeine 30 mg., and with alcohol 7.5 cc., caffeine 30 mg.; alcohol 10 cc. and caffeine in any dose was fatal, nor was death delayed with any dose.

Alcohol 7.5 cc. intraperitoneally was a surely fatal dose and caffeine in any dose did not retard the fatal outcome.

Conclusion. Alcohol and caffeine do not reduce the toxicity of each other in any dose; on the contrary, there is more than algebraic summation. Death occurs with less than one-half the fatal dose of each when given together; or when the sum of the percentage fatality is only 14.3 per cent, the combined fatality is 100 per cent. (The percentage fatality is the percentage of deaths with a given dose of alcohol or caffeine alone as deduced from this table; the combined fatality is the percentage of deaths with alcohol and caffeine combined).

To learn in how far the narcosis and motor depression of alcohol might influence the onset of the fatal symptoms in caffeine death, 200 mg. per kg. were administered to each of two animals in deep coma from alcohol 10 cc., but which had vomited, and to one animal less deeply narcotized. Death was delayed to three, five and twenty-four hours, (while with caffeine alone three experiments were fatal within twenty minutes). It seems therefore that alcohol narcosis delays death by caffeine, but the absolute fatality of caffeine is increased by alcohol, as was pointed out above.

All the caffeine animals died during or following convulsions; the alcohol animals in coma. In the "combined" death there may or may not be convulsions; with alcohol 4, + caffeine 60, four deaths were witnessed, of which two were without and two with convulsions; one animal with alcohol 4, + caffeine 30, died without convulsions. As with the caffeine alone, so with alcohol and caffeine combined cardiac failure seems to be the determining cause of death, judging by the frequency of irregularities, the cardiac dilation and the arterial condition of the blood post mortem.

Heart rate. As alcohol alone has little effect on the heart rate the results will be arranged and discussed in the order of the dosage of caffeine.

Caffein 2-5 mg. per kg.: Caffein up to 5 mg. has no effect on the heart rate; combined with very small (2 cc.) and very large (10 cc.) doses of alcohol the rate agrees with alcohol alone; with moderate (4 cc.) and large (7.5 cc.) doses the rate is considerably increased.

Alcohol—2 cc. per kg. Four experiments (including one with 10 mg.) of caffein. The rate was somewhat decreased agreeing with that of alcohol alone.

Alcohol—4 cc. per kg. Nine experiments. The rate was gradually increased to an average maximum from 30 to 35 per ten seconds.

7.5 cc. per kg. Three experiments. A somewhat greater increase in rate than with alcohol 4 cc. (from 29 to 37-8).

10.0 cc. per kg. Three experiments (one with 10 mg.). The rate was decreased as with alcohol 10 cc. alone.

7.5 cc. per kg. intraperitoneally. Four experiments. In two the rate was somewhat slowed during the day; in two there were irregularities and decrease in rate; this agrees with the alcohol.

Caffein—15 mg. per kg. Caffein 15 mg. has no effect on the heart rate; with very large doses of alcohol the rate again agrees with that of alcohol alone; with moderate doses the rate is unchanged and with larger doses is increased.

Alcohol—2.0 cc. per kg. No experiments.

4.0 cc. per kg. Two experiments. The average rate was unchanged except for a slight increase in twenty-four hours; one rate was slightly increased and one decreased.

7.5 cc. per kg. Two experiments. The rate was increased 5 to 6 during the day; two animals that vomited also gave an increased rate.

10.0 cc. per kg. Three experiments. The rate was decreased slightly for one hour and then returned to normal in two hours, and gradually became somewhat slower (animals died during the night).

7.5 cc. per kg. intraperitoneally. One experiment. The rate remained unchanged until shortly before the fatal slowing.

Caffein—30 mg. per kg. With caffein 30 mg. the rate is increased, with no irregularities; combined with alcohol in moderate doses and above, the heart frequently becomes irregular in rate and rythm; the rate may be increased or decreased, usually decreased with the larger doses.

Alcohol—2.0 cc. per kg. No experiments.

4.0 cc. per kg. Three experiments. Two animals had irregularity in rate and rhythm with one death in one hour. In the second, the rate was decreased from 40 to 21, 24, 27; a third gave a slight increase in rate; a fourth vomited but had an irregular heart.

7.5 cc. per kg. Two experiments. One animal with a decrease from 35 to 23 during the day, one animal with an increase from 32 to 41 during the day. Three animals vomited and all had a greatly increased rate at twenty-four hours although a lesser increase during the day; one had a very irregular heart.

10 cc. per kg. Two experiments. There was one irregularity with the rate otherwise unchanged; one with a decrease from 34 to 16 and 22, and death in five hours.

7.5 cc. per kg. intraperitoneally. One experiment in which the heart was unchanged in twenty minutes, with death in one hour.

Caffein—60 mg. per kg. Caffein 60 mg. increases the heart rate; with quite small quantities (2 cc.) of alcohol, the rate is greatly increased, more than with caffein alone; with larger quantities the effects are similar to 30 mg. with greater variations.

Alcohol—2.0 cc. per kg. Three experiments. The average rate was increased from 30 per ten seconds to an average maximum of 38 during the day; this is a greater increase than with caffein 60 mg. alone.

4.0 cc. per kg. Four experiments. The rate was irregular early; death within one and three quarters hours.

7.5 cc. per kg. Two experiments. The heart was irregular within thirty minutes but became regular shortly after; in one the rate increased maximally returning to normal in five hours; in the second there was a decrease from 38 to 25.

10.0 cc. per kg. Two experiments, in one the rate was greatly slowed and in the other was markedly irregular.

Caffein—120 mg. per kg. There were two experiments with alcohol 2.0 cc. in which early death prevented complete observations; in one the rate was maximally increased earlier than with caffein alone.

The cardiac effects of combined caffein and alcohol differ from those of either agent alone, and tend to be more deleterious. When the combination contains small quantities of caffein (to 5 mg.), with doses of alcohol which tend to quicken the rate (4

to 7.5 cc.), there seems to be some synergism; for the average rate is somewhat faster than with alcohol alone, although this dosage of caffein has no effect on the rate in the absence of alcohol; but it does not prevent the slowing from large (10 cc.) doses of alcohol.

When the dosage of caffein is increased to 15 mg. (which alone also has practically no effect on the heart rate) the synergistic effect on the quickening doses of alcohol is less marked; otherwise the phenomena are essentially similar to those of the 5 mg. doses.

With larger doses of caffein (30 mg. upward) the most conspicuous effect of the addition of alcohol (from 4 cc. upward) consists in the frequent and early occurrence of cardiac irregularities. The larger doses of alcohol also prevent the cardiac quickening which result when these doses of caffein are given alone. With still larger doses (60 to 120 mg.) even 2 cc. of alcohol tend to increase the rate and the irregularities which, with larger doses of alcohol, are a constant feature.

It would seem, then, that alcoholic intoxication renders the heart more susceptible to the deleterious cardiac actions of caffein, to a degree which could not be foreseen from the effect of the alcohol alone. This doubtless explains the increased fatality resulting from the combination of the two poisons.

Temperature. The effects of the two drugs, when given alone, appear to be opposite.

Alcohol alone (see fig. I, page 274) causes a loss of temperature, the extent and duration varying in the same direction as the dose.

Caffein alone (see table I) causes a rise of temperature, which is practically independent of the dose (0.5° with 5 mg. per kg.; 1.0 to 2° with 15 to 120 mg.).

With the two drugs together, the resultant effects are as follows:

(a) Small and moderate doses of alcohol (2 and 4 cc.) with small and moderate doses of caffein (2 to 30 mg.) give the same fall as alcohol alone. In other words, alcohol absolutely abolishes the caffein rise, while caffein has no effect, either positive or negative, on the alcohol fall.

(b) Large doses of caffein (60 mg.) counteract the fall of small doses (2 cc.) of alcohol constituting a limited antagonism.

(c) With large doses of alcohol (7.5 and 10 cc.) the addition of caffeine in all doses (from 5 mg. to 60 mg.) tends to increase the fall, so that the ordinary action of caffeine seems to be reversed. This agrees with the synergism in motor and psychic effects which is observed with the combination of this dosage.

Alcohol—2.0 cc. per kg. This produces a slight fall averaging 0.75° in two hours.

Caffein—2 and 5 mg. per kg. In two experiments the fall is the same as for alcohol alone.

10 mg. per kg. In two experiments the average fall was lessened 0.25° ; recovery about the same.

60 mg. per kg. In three experiments the alcohol fall was prevented and there was a rise of 0.4° in six hours.

Alcohol 4.0 cc. per kg. This produces a fall reaching its maximum in two to three hours, and ranging from 0.9 to 3.1° ; average fall 1.9° .

Caffein—2 mg. per kg. Two experiments increased the average fall slightly, but within the maximum limits.

5 mg. per kg. Seven experiments had little influence. In series "A," four experiments, the fall was 0.6° to 0.7° greater with caffeine; in series "B" three experiments, the fall was 0.6° to 0.7° greater in the controls; both within the maximum and minimum limits.

15 mg. per kg. Two experiments had no influence on the fall, but the recovery was somewhat earlier; within the maximum and minimum limits.

30 mg. per kg. Three experiments had no influence; within the maximum and minimum limits.

60 mg. per kg. Two experiments with early death and no temperature records.

Alcohol—7.5 cc. per kg. The average maximum fall was 2.8° ; the maximum in any experiment 4.0° ; the minimum 2.4° .

Caffein—5 mg. per kg. Three experiments increased the fall and delayed recovery; average maximum fall 4.7° ; maximum fall in any experiment 5.7° ; minimum 4.2° .

15 mg. per kg. Two experiments decreased the fall, with recovery not influenced; average maximum fall 2.2° with a lesser maximum and minimum.

30 mg. per kg. Three experiments increased the fall and delayed the recovery; average maximum fall 4.4° , maximum fall in any experiment 6.8° ; minimum fall 3.1° .

60 mg. per kg. Two experiments increased the fall; in five hours the fall was 5.5° and no recovery. The increased fall probably was due to the deeper narcosis of these animals.

Alcohol 10.0 cc. per kg. The average maximum fall was 3.8° ; the maximum in any experiment 7.5° ; the minimum 3.6° .

Caffein—5 mg. per kg. Three experiments increased the fall, with a maximum fall in any experiment 9.7° and a minimum of 5.3° .

10 mg. per kg. One experiment increased the fall with a maximum of 8.2° during the day.

15 mg. per kg. Three experiments had no influence; average maximum fall 4.2° ; maximum in any experiment 5.9° ; minimum 3.5° .

30 mg. per kg. One experiment decreased the fall; the maximum was 2.6° in two hours; a second experiment gave the same result in two hours when the animal vomited.

60 mg. per kg. Two experiments. The fall was practically the same; an average maximum of 3.7° in five hours; maximum and minimum in any experiment 3.8° and 3.7° .

Rate of respiration. Neither alcohol nor caffein, alone or combined, had any marked or constant effect on the respiration of the cats used in this investigation. Used alone, alcohol in all doses tends to increase the rate, most markedly with 4 cc.; caffein alone had no effect up to 30 mg.; higher doses showed slight quickening, increasing with the dose.

With the two drugs together, the resultant effects are:

(a) Small and moderate doses of alcohol (2 cc. and 4 cc.) with small and moderate doses of caffein (5 to 15 mg.) give the same slight quickening as alcohol alone. This is also true of small doses of alcohol (2 cc.) with large doses of caffein (60 mg.); and of toxic doses of alcohol (10 cc.) with small doses of caffein (5 mg.).

(b) Moderate doses of alcohol (4 cc.) and large doses of caffein (60 mg.), give a somewhat greater or more maintained quickening than alcohol alone.

(c) Large doses of alcohol (7.5 and 10 cc.) with moderate or large doses of caffein (from 10 mg. up) show less quickening than with alcohol alone; or the respiration may even be slowed.

In brief, the combination of large doses of either drug with small doses of the other gives practically the same effects as alco-

hol alone. The combination of a moderate dose of alcohol with a large dose of caffeine results in greater quickening. Large doses of alcohol show less quickening when caffeine is added.

Alcohol—2.0 cc. per kg.

Caffein—2, 5, and 10 mg. per kg. Four experiments; 60 mg., four experiments: the rate of respiration is practically unaltered.

Alcohol 4.0 cc. per kg.

Caffein—2 and 5 mg. per kg. Two experiments with 2 mg. and seven experiments with 5 mg. lessened the increased rate of alcohol 4.0 cc. somewhat. Occasionally, when an animal was excited, the rate increased greatly.

15 mg. per kg. One experiment caused no variation from the alcohol curve.

60 mg per kg. Three experiments in which the animals vomited. The increase was maintained somewhat longer than with alcohol alone; respiration was irregular in one experiment. In one experiment with early death the rate agreed with that of alcohol alone.

Alcohol 7.5 cc. per kg.

Caffein—5 mg. per kg. Three experiments. The increase in rate was somewhat more marked in degree and duration than with the average of the alcohol experiments although about the same as one control of the same day.

In the experiments with larger doses of caffeine the records are not complete; there is no increase in respiration, all the recorded rates gave either no change or a decrease.

Alcohol—10.0 cc. per kg.

Caffein—5 mg. per kg. Two experiments did not vary the alcohol curve; one experiment gave a slightly decreased rate and the second increased the rate as with alcohol 10.0 cc.

10 mg. per kg. One experiment in which the rate was increased as with one control but to a lesser degree.

15. mg per kg. One experiment in which the rate was increased as with one control but to a lesser degree.

30 and 60 mg. per kg. Two experiments; in each the rate at the second hour only was recorded; no increase in rate.

Alcohol 7.5 cc. intraperitoneally

Caffein—2 to 30 mg. In two experiments (caffeine 2 and 5 mg.) the respiration was practically the same as with alcohol alone; with caffeine 10 mg. (one experiment) it was unchanged; two experiments (caffeine 2 and 15 mg.) increased the rate to a maximum of 20 per fifteen seconds, but it returned to normal before death (which was not seen). In one experiment (caffeine 5 mg.) the respiration was observed to stop before the heart. With caffeine 30 mg. early death prevented observations.

The respiratory actions of caffeine, as described in the literature, are generally greater than those observed in this series. It may be that cats are less susceptible to the action of caffeine than are dogs or rabbits.

Impens (7) found that 0.020 and 0.050 gram caffeine in a medium sized rabbit increased the respiratory rate and volume slightly; with convulsive doses there was a great increase.

Heinz (7) —0.007 per kg. caffeine-natrio-salicyl in two rabbits increased the respiratory volume 40–45 per cent; 0.025 gram per kg. decreased the respiratory volume 7 per cent.

Binz (8) —0.15 gram caffeine to a dog narcotized with alcohol greatly increased the rate and depth of respiration without lessening the narcosis.

Cushny (2)—Caffeine quickens and strengthens respiration, more with alcohol than on normal animals.

Leven (9)—Caffeine increased the respiration in toxic doses in guinea pigs.

PART IV. SUMMARY OF THE EFFECTS

For the sake of brevity, the following descriptive equivalents of the doses (per kilogram) are used:

	ALCOHOL	CAFFEIN
	cc.	mg.
Small doses.....	2	2 to 10
Moderate doses.....	4	15 to 30
Large doses.....	7.5 and 10	60 and 120

1. *Psychic effects*

Alcohol produces narcosis, varying from sleepiness with small doses, light coma from moderate, and deep coma with large doses.

Caffein in non-fatal doses produces wakefulness and increased irritability; fatal doses, more or less excitement.

Caffein on the alcoholic narcosis. The alcohol narcosis is:

(a) Lessened somewhat when small or moderate doses of alcohol are combined with small or moderate doses of caffein (antagonism by algebraic summation of action).

(b) Intensified when moderate doses of alcohol are combined with large doses of caffein; or large doses of alcohol with caffein in all doses (synergism by reversal of caffein action).

Alcohol on the caffein excitement. The symptoms of caffein stimulation are too indefinite to justify conclusions. With large doses of alcohol the narcotic effects are always the more conspicuous.

2. *Reflexes*

Alcohol. Depressed reflexes and incoördination, increasing with the dose; the reflexes are abolished by large doses.

Caffein. Exaggerates reflexes, increasing with the dose.

Combined drugs. The results are similar to the psychic effects.

3. *Caffein convulsions*

Convulsions are only produced by fatal doses of caffein. They are not prevented by moderate doses of alcohol.

4. *Fatality*

The two drugs are always synergistic and the fatality is greater than simple summation. Death results by combining "small" doses of alcohol with "large" doses of caffein, and "moderate" doses of caffein with "moderate" doses of alcohol, but not by combining "small" doses of caffein with "large" doses of alcohol. In other words, the synergism is one-sided, alcohol increasing the toxicity of caffein, while caffein does not increase the toxicity

of alcohol. The death is always cardiac, suggesting that alcohol renders the heart more susceptible to caffein poisoning (one-sided synergism by increased susceptibility).

5. Heart rate

Alcohol. Small or moderate doses produce variable effects. Large doses slow.

Caffein. Small doses have small and variable effect; moderate doses cause considerable quickening; large doses give maximal quickening.

The rate is much increased by handling. No arhythmias were seen with caffein alone, even after large doses.

Combined drugs. (a) Small doses of both drugs cause greater quickening (synergism by summation).

(b) Moderate doses of alcohol lessen the quickening produced by moderate doses of caffein (antagonism by modified actions).

(c) Moderate doses of alcohol with moderate to large doses of caffein tend to produce cardiac irregularity (modified action).

6. Temperature

Alcohol produces a marked fall of temperature, increasing with the dose.

Caffein raises the temperature moderately; the dose has little influence.

Combined drugs. (a) With small or moderate doses of alcohol, small or moderate doses of caffein have no effect; large doses of caffein counteract the fall (antagonism by imperfect summation).

(b) With large doses of alcohol, caffein in all doses increases the fall (synergism by reversal of caffein-action).

7. Rate of respiration

Alcohol in our cats tends to increase the rate in all doses, most markedly with the "moderate" dose; the increase lasting 2 or 3 hours.

Caffein had no effect with small doses; progressive (but slight) increase with moderate to large doses.

Combined drugs. The effects of alcohol predominate.

(a) Large doses of either drug with small doses of the other, and moderate doses of both, give practically the same results as alcohol alone (synergism by imperfect summation).

(b) Moderate doses of alcohol and large doses of caffeine give more quickening than alcohol alone (synergism by summation).

(c) Large doses of alcohol show less quickening when caffeine is added (antagonism by reversal of action).

DISCUSSION OF THE RESULTS

BY TORALD SOLLMANN

Several practical conclusions can be deduced from the experiments, as will be mentioned later. Their deductions of more theoretical bearing are perhaps even more interesting. The combination of these two relatively simple drugs illustrates almost every conceivable variety of antagonism and synergism, as shown in the following schema:

	ANTAGONISM	SYNERGISM
<i>By Algebraic Summation of Actions;</i> (i.e., both drugs producing, qualitatively, their normal actions).	Narcosis (smaller doses) Motor system (smaller doses) Temperature (smaller doses)	Heart rate (smaller doses) Respiration (smaller doses)
<i>By reversed action:</i> (i.e., the actions of one or both drugs are the opposite of what they would be if used alone).	Respiration (larger doses) Heart rate (larger doses)	Narcosis (larger doses) Motor (larger doses) Temperature (larger doses)
<i>By increased susceptibility:</i>		Cardiac arrhythmia Fatality

With the smaller doses, each drug tends to act qualitatively as if it were present alone—though even here the effects generally appear to be modified quantitatively; with larger doses however, there is always a qualitative change in the direction tending to greater depression of functions. This tendency is the greater,

the larger the combined dose. In other words a "moderate" dose of the depressant can be antagonized only by a small or moderate dose of the stimulant; whilst large doses of the depressant render even small doses of the stimulant also depressant.

The combination of two stimulants results in summation of action only if both are used in relatively small doses; in larger doses they will become depressant. Evidently, the state of function produced by one drug modifies the response to other drugs, not only quantitatively, but also qualitatively.

It would not be justifiable of course, to extend these empirical facts to other drugs, until more work has been done. It is suggestive, however, that these facts agree essentially with those observed in the simultaneous action of pilocarpin and atropin on the development of embryos of the sea-urchin and the star-fish (10).

In the latter case, the drugs presumably have the same point of attack in the cell, whilst caffein and alcohol presumably act on different mechanisms. The agreement suggests therefore, that these empirical facts may be more or less general laws.

CONCLUSIONS

1. The response of cats to alcohol, to caffein and to combinations of the two, are summarized on pages 293-5.

2. When small doses of caffein and of alcohol are combined, the result is generally a qualitative algebraic summation of both actions, i.e., each drug produces, qualitatively, its ordinary effects.

3. When large doses of the two drugs are combined, the effects of the stimulant drug tend to be reversed, resulting in greater depression.

PRACTICAL DEDUCTIONS

1. *Caffein in alcohol poisoning.* With fatal doses of alcohol caffein acts only deleteriously; with half fatal doses, moderate doses of caffein may decrease the narcosis and hasten recovery; large doses are dangerous.

2. *Alcohol in caffein poisoning.* With small doses of caffein,

alcohol lessens the psychic effects; with large doses, alcohol adds to the danger.

3. *Caffein*. The danger of cardiac death is increased by agents which alone have relatively little direct depressant effect on the heart. This would engender caution in the use of *caffein* in heart disease.

In conclusion, I wish to express my grateful appreciation to my chief, Professor Sollmann, for his many suggestions throughout the course of the experiments, and for correcting and revising the manuscript.

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PHYSIOLOGICAL STUDIES IN ANAPHYLAXIS

IV. REACTION OF THE CAT TOWARD HORSE SERUM

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As a rule the cat's reaction to intravenous and subcutaneous injections of horse serum resembles that of the dog, differing chiefly in intensity. Its reaction may also resemble that of the rabbit in many respects, showing, however, grave vascular, while the rabbit shows grave cardiac symptoms. Thus far it has not been definitely proved just how the foreign protein causes the death of cats, but it is generally thought it may be through dilatation of the blood vessels as a result of vasomotor paralysis. It seems to me that in discussing protein intoxication those who attribute death of sensitized and non-sensitized animals to the paralysis of the vaso-motor nerve endings or of smooth muscle of the blood vessels, have overlooked certain other important factors. Indeed they have not even proved that the fall in blood pressure is due to vaso-dilatation. It is hoped that in this discussion it will be shown that even with a very circumscribed circulation area it is possible to obtain a fall of blood pressure and even to kill the animal under conditions that preclude vaso-dilatation as the chief cause. Furthermore it is hoped to show by eliminating distant influences, such as impulses from the central nervous system or the action of substances formed in the liver or intestine, that for the organs involved, the causal factors are for the most part peripheral. To this end it is desired to consider some experiments with horse serum in which is studied the reaction of:

1. Intact animals non-sensitized, non-anesthetized (except with cocaine at point of inserting the cannula).
2. Anesthetized animals (sensitized and non-sensitized) as indicated by blood pressure records and by cardiograms.
3. Animals after vagotomy, atropin, and after destruction of the central nervous system.
4. Animals with the splanchnic or brachial circulation eliminated.
5. The systemic blood vessels when perfused.
6. The pulmonary circulation and of the isolated heart when perfused.

1 REACTION OF THE INTACT ANIMAL

The gross symptoms of serum intoxication in the cat are practically the same as those observed in the sensitized dog, and for the latter animal have been quite accurately described by Pearce-Eisenbrey and others. If one inject cats intravenously with sterile horse serum, grave symptoms result, both in non-sensitized, and in sensitized individuals, the difference between the macroscopic reaction of the former as compared with that of the latter is chiefly one of degree. Twenty-five ten-thousandths of a cubic centimeter of horse serum per gram body weight injected into the jugular vein of young cats is nearly always fatal providing the total dose be injected steadily in from ten to twenty seconds. Adult cats quite frequently, as will be seen in the following protocol, recover from 0.0020 cc. to 0.0025 cc. per gram body weight, but suffer great depression which lasts forty-five to sixty seconds. This reaction ensues with normal serum and with serum heated 30 minutes at 56°C. when the injection is continuous.

Protocol 1. Non-sensitized cat

Normal female cat, weight 2994 grams, cocaine local anesthesia, otherwise no drugs used. Cannula in left saphaenus vein.

12:31 p.m. Six cubic centimeters of warm sterile horse serum injected slowly and continuously into the left saphaenus vein; injection interval about forty-five seconds, struggled during first part of injection, head

dropped limp about ten to fifteen seconds before injection was completed, animal showed muscle weakness before the cannula could be removed and the animal placed on the floor.

12:32 Lying limp on floor, unable to walk, pulse very thready.

12:33 Feces passed; character of respiration changed, rate about 102 per minute as compared with about 50 per minute before the injection. Respirations were slightly dyspnoeic, after each series of 15 to 17 there was a deep inspiration, head on floor, mouth partly open, pupils constricted to a mere slit even in dim light.

12:34 One hundred and twenty respirations per minute, mouth open as if panting, some salivation, spreading of claws and weak clawing towards mouth as if to remove something, conscious of dog's presence, weak ineffectual effort to get upon front feet.

12:36 Head slightly raised, mouth open as if panting for breath. From now on the cat improved slowly so that by 1:04 p.m. she was able to walk a little.

4:30 Respirations nearly normal, able to walk but prefers to lie down. Cat recovered, and two weeks later gave birth to five healthy kittens.

2 THE REACTION OF ANESTHETIZED ANIMALS AS INDICATED BY BLOOD-PRESSURE RECORDS AND BY CARDIOGRAMS

In this series of experiments the cats were as a rule anesthetized with ether, sometimes with chloral, urethane, and ether. The depth of anesthesia has some influence in determining the dose of serum necessary to kill, consequently during operation the animal was deeply anesthetized, and before the injection the dropping bottle was adjusted so that light anesthesia was maintained. The blood pressure was recorded from the carotid artery by a mercury manometer, and the right ventricle recorded its contractions by the upstroke of a Cushny myocardiograph.

In the intact animal it was stated that there was pronounced muscle-weakness, a thready pulse, and polypnoea. When records of the carotid blood pressure and of the right ventricular beat are taken simultaneously, it is easy to see why injections of horse serum cause such remarkable phenomena of collapse. Even before the intravenous injection is completed there is noted a change in the heart beat, and following the slight preliminary rise of

blood pressure there is a rapid fall thereof. With such cats as are unusually sensitive to ether, even 0.0010 cc. per gram body weight sometimes causes death, but ordinarily two-thousandths of a cubic centimeter per gram body weight, injected into the jugular vein in five to ten seconds causes the following phenomena. The effect upon the heart will depend somewhat upon the condition of this organ and upon the amount of blood in it. With a low blood pressure and a weak heart there is a slight rise of pressure accompanied by a strengthening of the ventricular contractions; with the blood pressure about normal there is a decrease in the amplitude of the beat and some irregularity of rhythm as soon, apparently, as the serum enters the right heart, as if the serum in some way interfered with the proper conduction of the auriculo-ventricular muscle; following this and simultaneously with the rapidly diminishing amplitude and rate of beat, there is a corresponding fall of blood pressure. The right auricle is gorged with blood, the right ventricle is greatly distended, the blood gradually becomes more and more dark, and the left auricle and ventricle are practically empty but continue to beat regularly for some time after the right heart has become greatly distended. In some cats the serum not only causes a change in rate of the heart, but it also causes more or less incoördination thereof. Inspection of such a heart sometimes reveals hemorrhagic blotches on the surface of the ventricle. Sooner or later the right auricle and right ventricle beat with an independent rhythm, and the latter sometimes passes into fibrillary contractions from which it seldom recovers. Long after the ventricles have ceased to record their contractions and show nothing but mere twitchings the auricles continue to beat quite strongly and regularly. (See Fig. 1.)

Protocol 1. Anaphylactic Experiment 303

Black and white female cat. August 9, 1911, received a subcutaneous injection of 2 cc. of horse serum. Fed upon cooked beef, cow's milk, and water. November 21, 1911, weight 3575 grams, fat and healthy. Ether anesthesia. Injection cannula in right external jugular vein, blood-pressure cannula in right carotid artery. Intrapleural cannula in chest cavity about level of base of ventricle.

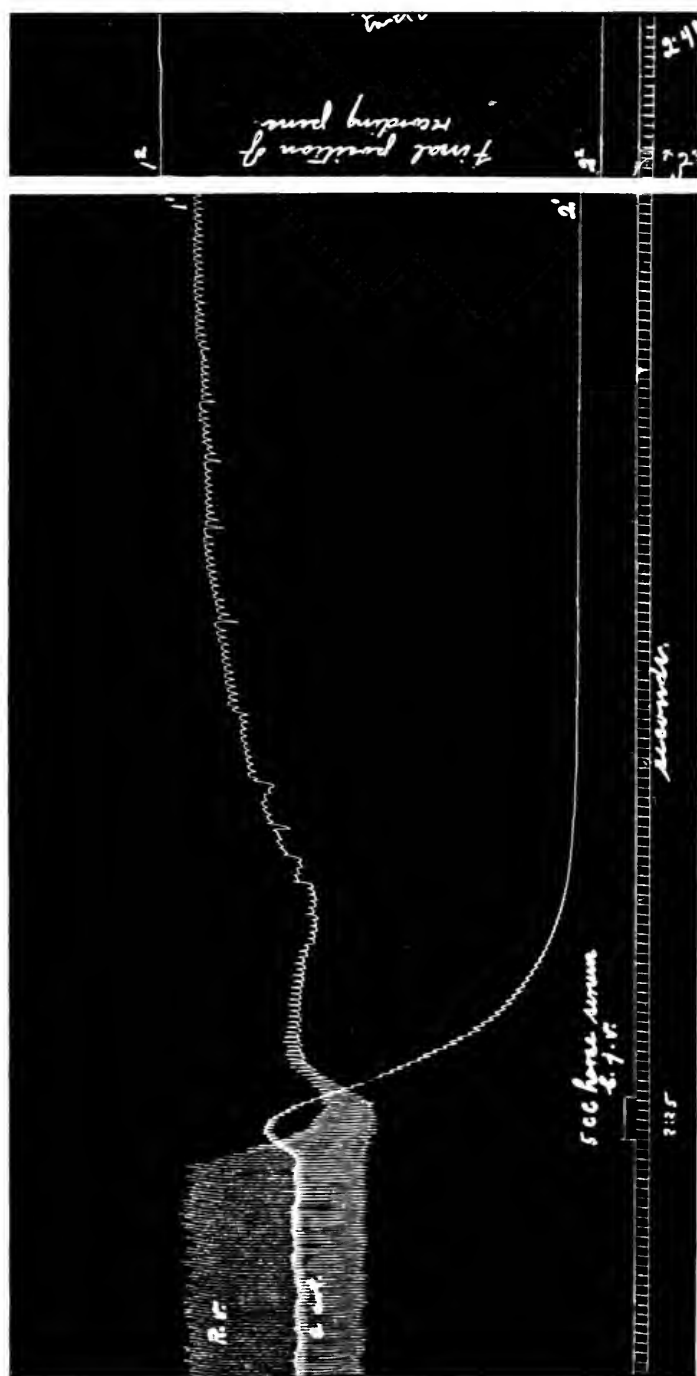


FIG. 1. SHOWING THE EFFECT OF HORSE SERUM UPON THE HEART AND BLOOD PRESSURE OF CAT

Experiment 94. Normal cat. Weight about 2½ kilograms. *a*, Blood pressure recorded from right carotid artery by a mercury manometer. *R. v.* Myocardiogram of right ventricle, upstroke represents systole. Modified Cushman-myocardiograph used. Five cc. normal horse serum injected into left jugular vein in about 4½ seconds. Cut reduce 1 to $\frac{7}{10}$ of the original.

4 p.m. Animal under light ether anesthesia, 0.0020 cc. per gram body weight or a total of 7 cc. of warm horse serum injected into the right external jugular vein in about twenty-five seconds. Before one-half the total volume of serum had been injected the blood pressure suddenly fell, the chest walls remained fixed in a semi-inspiratory stage, and judging from the respiratory record the lungs filled the chest cavity and were arrested in the inspiratory state. After a fall in blood pressure of about 60 mm. a series of respiratory gasps were made; the blood pressure rose a few millimeters after which it slowly fell to zero.

There is then in the sensitized animal a striking difference between the irritability of the cardio-respiratory apparatus. The lung of the sensitized cat, however, differs from that of sensitized guinea-pig in that when the chest cavity is opened the lungs of the cat collapse much more completely. There is only temporary and partial immobilization in the inspiratory stage. The excised lung of the sensitized cat collapses nearly as completely as does the excised lung of an asphyxiated cat. The heart of the sensitized cat reacts much in the same manner as does the heart of a sensitized guinea-pig.

Upon opening up the heart one may or may not find traces of a blood clot, depending upon the length of time intervening between the cessation of respiration and the moment of examining the heart. In most of the animals examined soon after the blood pressure had fallen to zero no clot was found. Judging from the pliability of the tissues the muscle of the right ventricular wall seemed to pass into rigor at about the same time as that of the left heart, and in some cases perhaps a little sooner. In this respect there seems to be an additional difference between the reaction of non-sensitized and of sensitized animals. Greater emphasis will be laid upon the detailed differences in a subsequent paper now in the process of completion. (See fig. 2.)

3 THE REACTION OF ANIMALS AFTER VAGOTOMY, ATROPIN, AND DESTRUCTION OF THE CENTRAL NERVOUS SYSTEM

a Effect of vagotomy

In my earlier experiments the right and left vagi were either tied off or severed, but this seemed to have little influence upon the end results, and in the light of later experiments it is perfectly clear that sectioning these nerves should not influence to any



FIG. 2. SHOWING THE EFFECT OF HORSE SERUM UPON THE INTRAPEURAL PRESSURE AND UPON THE BLOOD PRESSURE OF THE CAROTID ARTERY

Experiment 303. Sensitized female cat. For details see protocol No. 2. *A*, Base line representing blood pressure zero. Time in seconds. *B*, Blood pressure of carotid artery, injection of 7 cc. of serum begun at (*a*) and completed within about 25 seconds. At (*x*) and (*xx*) rubber connexions pinched to dislodge a small clot in the blood-pressure cannula. The blood-pressure pen should have followed the course of the dotted line and reached its lowest level in about 2½ minutes after (*x*) or a few seconds over four minutes after the beginning of the injection. *C*, Record indicating changes in the intrapleural pressure. *cdc*, final respiratory gasps. *hc*, period of inspiratory immobilization of the lungs; record exaggerated by filling up of the large thoracic veins and of the right heart with blood.

marked extent the toxic action of serum. As will be pointed out more conclusively later, central impulses have but little to do with the phenomena herein described. Auer states that in the guinea-pig even degeneration of the vagi do not influence the results; my results so far confirm his findings.¹ It is even doubtful if the peripheral nerve endings play a very important part in causing the muscle-elements to respond as they do, the only data that even suggests that they might is the reaction observed in animals treated with large doses of atropin.

b Atropin sulphate

Of late a great deal has been said about the prophylactic action of atropin sulphate when used in connexion with horse serum anaphylaxis. Some have even gone so far as to see a quantitative relationship between the action of horse serum and that of atropin sulphate. This drug has some action in hindering the usual action of horse serum and for reasons which will be pointed out later, it is supposed to be only apparently quantitative. Anderson and Schultz stated in an earlier paper that it may utterly fail to neutralize the stimulating action of the serum, and at best is a rather uncertain remedy in immediate anaphylaxis of the guinea-pig. With cats, doses of atropin that completely paralyze the vagus endings to electrical stimulation and that dilate the pupil for a considerable time, do not neutralize the action of the serum since the cats die recording the same cardiac and vasomotor phenomena as recorded by non-atropinized animals. With larger doses the results are uncertain. In some animals an intravenous injection of one milligram of atropin per kilo results in recovery, in others it apparently has no action in retarding the fatal action of the foreign protein. For a cat weighing 5 kg., 5 mg. of atropin sulphate injected into the jugular vein may cause a fall of blood pressure and greatly diminish the force of the ventricular contraction. Increasing the dose of atropin intensifies these conditions, the contractility of both cardiac and smooth

¹ My experiments on this particular phase of the subject are still in progress and the results will be published later.

muscle is reduced, and naturally any substance that ordinarily stimulates such muscle to contract will be hindered from doing so by the toxic action of the atropin. Atropin, then, it would seem, like chloral, is primarily of academic interest and of little practical value in treating cases of anaphylactic shock. Even the academic interest is dulled because of the ineffectiveness in experimental anaphylaxis of minimum doses known to paralyze the nerve endings of the vagus and the motor nerves of the iris. Even a dose of atropin sulphate one hundred times that required to paralyze the motor nerves of the cat's iris has practically no influence upon the action of 0.0025 cc. of horse serum per gram body weight. As will be seen by the following protocol and fig. 3, the animal died just as if no atropin had been given.

Protocol 3

Experiment No. 95. Normal, non-sensitized ♂ cat. June 12, 1911. Weight, 2395 grams. Ether anesthesia. Blood pressure by mercury manometer from right carotid artery. Cannula in left jugular vein. Window in chest and cardiogram recorded by a Cushny myocardiograph, upstroke represents systole of the right ventricle.

11:52+ Amplitude of cardiogram = 36, rate of contraction = 195, blood pressure = 150 mm.

11:54 Five milligrams (1 cc.) atropin sulphate injected into left jugular vein.

Eight seconds after beginning the injection, amplitude of cardiogram = 28, rate of contraction = 195, blood pressure = 152 mm.

About one minute after the injection, the amplitude of the cardiogram = 23.5, rate of contraction = 200, blood pressure = 114 mm.

The blood pressure then fell about 4 mm. lower, and later gradually returned to the higher level as the ventricular contractions grew slightly stronger.

11:59+ Amplitude of cardiograms = 35.5, rate of contractions = 200, blood pressure = 118. The injection of serum caused a rise of 12 mm. "injection rise," after which the blood pressure fell to zero in the course of about one and a half minutes. The right ventricle gradually filled up in the same time, and its contractions grew gradually weaker until the lever no longer recorded them.

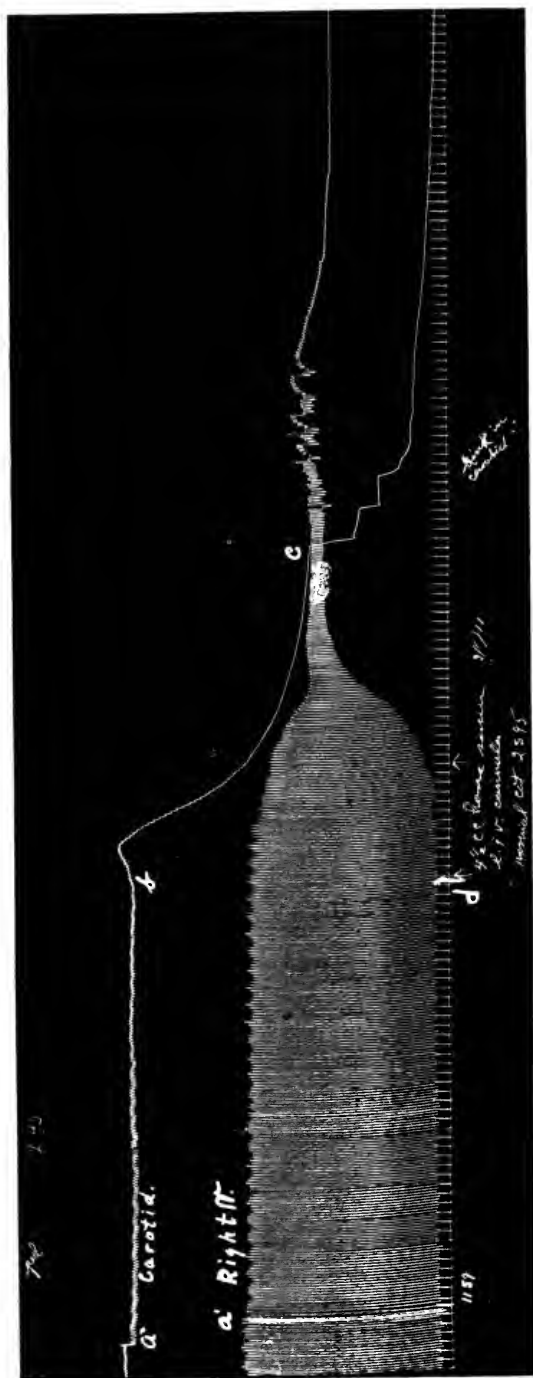


FIG. 3. THE EFFECT OF HORSE SERUM UPON THE HEART AND BLOOD PRESSURE OF A CAT TREATED WITH ATROPIN SULPHATE
Experiment 95. June 12, 1911. See protocol I, p. 9, for details. Right carotid blood pressure recorded by mercury manometer. Pens not in line sec (a) beginning of record. Owing to a slight kink in the cannula the blood pressure curve at (c) is step-like.

While it must be admitted that such substances as atropin sulphate, chloral hydrate, ether, etc., may alter the reaction towards serum and save certain animals from acute anaphylactic shock, yet these substances have to be used in large doses, indeed, in such large doses that they probably greatly lessen the cells' irritability long after the irritability of the nerve endings has been lost, so that not only serum, but also many other stimulating substances act but slightly. The action of atropin stands thus: toxic doses, that actually poison the cell protoplasm, thus reducing its irritability, leaving the cell in a semi-moribund condition, may or may not save the animal from immediate anaphylaxis. Doses of atropin, however, that do not greatly reduce the cells' irritability but do paralyze the motor nerves of the vagus and of the iris, have no apparent antagonizing effect upon serum intoxication. This favors the hypothesis that serum acts primarily upon the receptive substance of the cell itself or in some way affects certain physico-chemical changes in the cell.

c Destruction of the central nervous system

In these experiments a window was cut into the chest, the trachea dissected free from the mass of blood vessels, and the latter clamped by means of a special clamp. The neck muscles were cut away and finally after slightly raising the cervical vertebra, the head was removed. The spinal cord was then destroyed, if desired, and the cut end of the vertebra covered with oiled cloth or vaselined cotton. This operation can be accomplished easily in less than an hour's time, with the loss of but a few drops of blood.

As is well known such preparations have a low blood pressure and a weak heart. The blood pressure can be raised by perfusing fresh blood from a second animal or the aorta can be clamped off, in which case there is a sharp rise of blood pressure, and a strengthening of the heart beat. As will be shown later this artificially high pressure is not permanent, but gradually falls to a lower level. If now a lethal dose of serum be injected there is a sudden fall of pressure as before, and a corresponding weakening of the ventricular contraction. Indeed, except for the low initial blood

pressure no essential difference can be detected between the reaction of such an animal and one with the central nervous system intact. In the cat, then, it is safe to conclude that the nervous impulses from and by way of the central nervous system are not essential factors in causing death in serum intoxication or in anaphylactic shock. Since the cause is not to be found in the central nervous system it must be in some part of the respiratory or circulatory system. But the lungs of the cat do not show signs of bronchial spasm as is observed in the guinea-pig, at least there is not that permanent distension of the lung tissue as has been described by Auer and Lewis, Anderson and Schultz, Biedl and Kraus, and more in detail by Schultz and Jordan. Respiratory symptoms, it is true, are observed both in the intact and in the operated animal, but they are somewhat obscure, as will be shown directly in connexion with animals in which the circulation of the splanchnic and head regions have been practically eliminated.

4 THE REACTION OF ANIMALS WITH THE SPLANCHNIC OR BRACHIAL CIRCULATION ELIMINATED

In this series of experiments I have endeavored to eliminate, as far as possible, that part of the circulation heretofore held to be responsible for the fall in blood pressure. It has been so common to attribute a fall in blood pressure to splanchnic vaso-dilatation that almost every obscure fall of pressure is now said to be due to dilatation of these vessels.

After having exposed the heart to record the contractions of the right ventricle, in one set of this series of experiments the descending aorta and the ascending *vena cava* were clamped close to the diaphragm on the thoracic side, and in the other set an additional clamp was used to cut off all of the blood vessels going to and coming from the anterior portion of the body—that is, the clamp included the vessels just above the aortic arch so that when all three clamps were closed the circulation area was practically limited to the lungs and that region supplied by the vertebral branches of the thoracic aorta. This was demonstrated by injecting such a preparation with colored gelatin.

As is well known, clamping the abdominal aorta causes a rise of blood pressure. Likewise clamping the thoracic aorta and inferior *vena cava* just above the diaphragm causes a rise of blood pressure, which may exceed the pressure just before clamping by three fold. Since after clamping there is some leakage of blood into the abdominal cavity by way of the vertebral arteries, an exudation of the more liquid portions of the blood into the tissue spaces, an adjustment of the circulation area of the lungs, and a greater distension of the right heart, this very high blood pressure is not maintained, but tends to return to the level just before clamping the aorta. An injection at this time into the jugular vein of 0.0025 cc. of serum per gram body weight usually kills the animal. The heart is at first slowed, and after a brief period of incoördination the right ventricle beats with greater force, but in spite of this the blood pressure in the carotid falls. The right auricle and ventricle both gradually fill, become greatly distended while the contractions of the latter grow weaker and weaker; the left auricle from the time that the blood pressure falls is small and empty, likewise the left ventricle seems to be empty, but they continue to contract rhythmically and coordinately for some time after the right heart fails to record its twitchings. Sometimes the lungs shortly after an injection of serum seem not to respond so readily to the inflating action of the bellows, but when a slightly higher intratracheal pressure is used the more dorsal lobes inflate, but are whiter than before. After death, however, they collapse as in non-serum treated animals. The ventricles soon cease to do naught but twitch, the auricles continuing to contract strongly and regularly for some time after the right ventricle shows only fibrillar contraction and has ceased to make a record. Although these experiments do not prove that the splanchnics, following a lethal dose of serum, take no part in the fall of blood pressure usually observed in normal and sensitized cats, they show that death may result without their influence, and that the phenomena observed are practically the same as when the splanchnic circulation is intact. Certainly the fall of blood pressure cannot be attributed to extra cardio-pulmonary causes alone. In spite of the contentions of Arthus, Friedberger,

Biedl and Kraus, Pearce and Eisenbrey, Auer, and nearly every writer on this subject that horse serum causes vaso-dilatation, it can be shown to cause vaso-constriction. If this be so there is no reason for hesitating to say that the heart and lungs are for the most part responsible for the low blood pressure, and that the venous congestion observed is for the most part a passive effect brought on by a block in the circulation, making it impossible for the right heart to empty itself.

By reason, then, of the number of possible factors involved in producing the above picture it becomes necessary to study the reaction of the different parts of the circulation. If serum stimulates smooth muscle to contract, it should cause vaso-constriction in the systemic arteries, but in the intact animal contraction of the coronary and the pulmonary arteries may also happen, in which case the rise of blood pressure that should occur because of the constriction of the systemic vessels would be neutralized and wholly masked by the diminished amount of blood entering the left heart because of the pulmonary resistance or because of the decreased output by the right ventricle. Therefore, in order to analyze more in detail the causal factors involved, the systemic system, then the coronary, and finally the pulmonary systems were perfused and their relative outflow measured before and after treatment with horse serum.

5 THE PERFUSION OF THE SYSTEMIC SYSTEM

In this series of experiments the cats were anesthetized with ether. Some of the animals were bled from an artery and Ringer was run into a vein until the blood was washed out of the tissues and they appeared clear and white, others were perfused at once. A cannula was then tied into the descending aorta just above the diaphragm, the head pressure being about 10 inches of the solution used. A second cannula was tied into the *vena cava* at the same level, the outlet being about 3 to 4 inches below the body of the animal. Such preparations always sooner or later take up and retain considerable Ringer solution, so that the tissues surrounding the pancreas, the stomach, intestines, etc., becomes distended

even to the extent of bulging out the wall of the abdomen. This condition may be brought on sooner by using higher pressures, indeed within certain limits the rate of becoming oedematous is more or less proportional to the pressure used. The temperature also has something to do with the rate at which the tissues retain the perfusing fluid, being hastened as the temperature is raised (20° to 40° C.).

In spite of the tissues taking up a certain amount of solution it could easily be shown that each of such substances as serum, barium chloride, epinephrine, and sodium nitrite, has a characteristic action when added to the perfusing solution.

The best results are obtained with freshly prepared animals, when the arterial walls, unless already constricted, are most irritable. Yet it is also possible to obtain very striking and consistent results from preparations having been perfused until the tissues are decidedly oedematous and the abdominal wall is bulged with retained fluid. Measured by a Wigger's perfusionometer it was found that a young cat weighing 670 grams, perfused into the aorta with Ringer at a pressure of 10 inches, two 50 cc. volumes were collected from the *vena cava* in gradually increasing rates of 115 and 106 seconds, respectively, then a solution containing 20 cc. of horse serum and 80 cc. of Ringer was turned on, the first 50 cc. volume was collected in 131 seconds and the second in 150 seconds. Though this experiment may be classed as one showing medium constriction the reduction in the caliber of the vessels was such as to decrease the outflow 30 or 40 per cent. It is evident, therefore, that horse serum constricts the blood vessels of the systemic circulation even at relatively low temperatures and pressures. In the blood pressure experiments there must then be some reason for the mercury manometer not recording a rise of blood pressure in spite of this action of serum upon the systemic blood vessels, for there is no apparent reason why these vessels should act differently in the perfused and intact animal. So the pulmonary vessels were studied by similar methods to see what possible influence they might exert upon the blood pressure when treated with serum.

6 REACTION OF THE PULMONARY CIRCULATION WHEN PERFUSED

As is well known, the excised lungs perfused with Ringer solution at body temperature shortly become quite oedematous. Nevertheless in the lungs of the cat I have been able to demonstrate with ease and beyond a doubt that the blood vessels constrict in response to serum, barium chloride, and epinephrine, and to dilate when perfused with NaNO_2 .

Young cats were given ether, a cannula tied into the pulmonary artery, the lungs were washed free from blood, then excised and perfused slowly with Ringer solution at 28° to 30° C., at a pressure of 10 inches of Ringer. In some experiments the lungs were rhythmically inflated by an artificial respiration apparatus, in other experiments the artificial respiration was omitted. Since it is primarily the action of the serum with which I am at present concerned, the discussion will be limited to the lungs perfused in the condition of collapse like that immediately after removal from the chest cavity, the influence of rhythmical inflation being left for future discussion. If a continuous series of perfusions into the pulmonary artery be made with Ringer alone, a gradual change in the rate of flow is observed, there being at first a slightly increased, and later a gradually diminishing output. If after perfusing from 100 to 200 cc. of Ringer from pressure bottle A, one quickly changes and perfuses from bottle B, containing for each 100 cc. of solution, 20 cc. of horse serum, there is a sudden drop in the output from the lungs. The following protocol illustrates more in detail a single experiment:

Protocol 4

Experiment July 5, 1911. Young ♂ cat, non-sensitized, weighing 900 grams. Ether anesthesia, nearly to point of respiratory failure, chest opened, cannula placed in right pulmonary artery, right auricle cut open, cannula connected with two constant pressure bottles, A containing Ringer, B containing diluted horse serum, 20 cc. serum and 80 cc. Ringer, A and B 10 inches above the perfusion cannula. Each perfusion period represents 50 cc. of solution, measured and recorded by the method described by Wiggers.

11:00 Perfused 50 cc. in 113 seconds.

11:02 Perfused 50 cc. in 98 seconds.

11:04+ Twenty per cent serum turned on after the perfusion of about 15 cc. of Ringer, perfusion period 110 seconds.

11:05+ Next 50 cc. required 192 seconds or nearly twice the time required to perfuse the Ringer alone. Changed to Ringer.

11:40+ Injected 1 cc. of 10 per cent NaNO_2 into rubber connexion, about in middle of perfusion period greatly increased rate, period covering 169 seconds.

11:53+ This perfusion period would have taken about 120 seconds, but 1 cc. of 10 per cent BaCl_2 was injected into the rubber connexion, perfusion rate very slow, the 50 cc. period not being completed until the end of 257 seconds.

SUMMARY AND CONCLUSIONS

It is evident from what has been said that horse serum is not such a harmless and inert substance as was formerly supposed. Even in the normal animal it acts quite generally upon such organs as are well supplied with smooth muscle. Thus in some intact animals the intestines are stimulated to marked peristalsis, and when containing feces the large intestine expels it. *In vitro* the intestinal segments are either stimulated to more rapid rhythmic movements or are thrown into tonic contraction; the bladder if full in the intact animal, is emptied; if excised and suspended in Ringer and treated with the proper concentration of serum it contracts. The smooth muscle of the eye is also affected, as will be discussed later. The uterus of an intact animal is thrown into tonic contraction and when excised is stimulated by serum, somewhat like the intestine. The arterial system as shown by perfusion experiments constricts in response to relatively concentrated solutions of serum. The pulmonary circulation, when perfused, also constricts in response to serum and finally the excised heart when perfused by Langendorff's method, also shows a diminished output if serum is added to the circulating media. One is then justified in saying that all muscle that has the general physiological properties of intestinal muscle, reacts towards serum by contracting.

Since smooth muscle in general contracts, or is stimulated to greater action by serum, it is difficult to accept the common explanation for the fall of blood pressure always observed in connexion with anaphylactic shock and with toxic doses of proteins, for as is generally held the foreign protein paralyzes the vasomotors and causes vasodilatation. The truth of the matter is that in the cat the circulatory disturbance is not due primarily to the splanchnics; instead the primary causes are factors which result in a block in that part of the circulation occupied by the right heart and pulmonary arteries. One of these factors is the increased resistance offered by the constricted pulmonary arteries, and a secondary factor is the action of the serum and the effects of over distension upon the right ventricle. If this is true then, unless the pulmonary constriction is too patent, gentle massaging of the right heart before fibrillation sets in ought to force enough blood past the block to tide the tissues over until the vascular muscle relaxes sufficiently to admit of the right ventricle performing its function. Indeed, this is sometimes possible and I have revived several animals in this way. This is also in accord with results described in an earlier paper, in which it was pointed out that even sensitized muscle sooner or later relaxes in the presence of serum, so that if a second dose of serum be introduced into the suspension fluid, the excised muscle again contracts, but usually with less force than in response to the first treatment. So also in the intact animal, one or two hours after recovery from serum shock, a second injection, though slightly larger than the first, usually has less action in both non-sensitized and sensitized cats. If, therefore, vaso-dilatation plays any part at all it must be after the serum has caused vaso-constriction of the coronary and pulmonary arteries, and possibly after systemic vaso-constriction at a time corresponding to the period of relaxation of smooth muscle when in contact with the foreign serum. It is possible that this return of smooth muscle to normal along with any extra dilatation on the part of the blood vessels might aid in further lowering the blood pressure, but certainly it is not an important factor in causing the preliminary fall of pressure. The cause rather lies in the two factors already mentioned.

In conclusion, then, one might summarize the most important facts as follows:

1. Horse serum causes constriction of the pulmonary arteries, coronary arteries, and of the systemic arteries, and also acts directly upon the heart-muscle.

2. In both normal cats and in cats sensitized with horse serum there is a fall of blood pressure, following an intravenous injection of 0.0010 to 0.0025 cc. of horse serum per gram body weight. The right auricle and right ventricle become gorged with blood, the pulmonary arteries are likewise greatly distended, whereas the left heart is practically empty. Non-sensitized cats very often recover from an intrajugular injection of 0.0025 cc. of horse serum per gram body weight. Highly sensitized cats usually die suddenly after intrajugular injections of 0.0020 and even 0.0010 cc. of horse serum per gram body weight.

3. The action of the serum is peripheral, since destruction of the brain and spinal cord does not materially alter the end results. Furthermore, perfusion studies as well as other studies with excised organs yield results that show smooth muscle and cardiac muscle to be the most important tissues involved in causing the gross symptoms of anaphylactic shock observed in the cat and dog.

4. The abdominal blood vessels play at best only a secondary part in causing the low blood pressure since a fall of blood pressure is obtained when all abdominal vessels are clamped off.

5. Atropin sulphate may or may not influence the circulatory phenomena. It is only very large doses of atropin that seem to have any influence upon the action of serum when the protein is intravenously injected in doses of 0.0010 to 0.0025 cc. per gram body weight.

6. Horse serum kills cats, then, by its action upon the cardio-pulmonary system and not by vaso-dilatation of the systemic blood vessels alone the distension of the large abdominal and thoracic veins being for the most part passive. The rate of injecting the serum into the jugular vein, as well as the total amount injected at once, greatly influences the blood pressure and cardiac phenomena, large doses rapidly injected being more certainly fatal.



TWO CRYSTALLINE PHARMACOLOGICAL AGENTS OBTAINED FROM THE TROPICAL TOAD, *BUFO AGUA*¹

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CONTENTS

I. Historical and general.....	320
Experiments made with the crude poison.....	325
II. On the presence of dihydroxy-methyl-amino-ethylol-benzene in the secretion.....	327
Physiological action of this substance.....	331
Percentage amount of this substance in the venom.....	333
The chromaphil reaction of the poison glands.....	334
III. On Bufagin, a crystalline compound and methods for its isolation and purification.....	334
IV. Chemical properties of Bufagin.....	337
Color reactions.....	337
Analyses.....	339
Behavior toward bromine and other reagents.....	341
Determination of its molecular weight.....	342
Possible relationship to cholesterin.....	343
A brief consideration of the earlier chemical investigations on toad venom.....	344
V. Pharmacological action of Bufagin.....	346
A. Action of Bufagin on cold-blooded animals.....	347
Experiments on frogs and terrapins.....	347
Experiments on the toad, <i>Bufo Agua</i>	348
B. Action of Bufagin on warm-blooded animals.....	356
Effect on the intact animal and on man.....	356
Effect on animals under anaesthesia.....	360
1. General action on the circulatory apparatus.....	360

¹ The subject matter of this paper appeared as a preliminary communication entitled: "The Poisons of the Tropical Toad, *Bufo Agua*," in the Journ. Americ. Med. Assoc., May 27, 1911, vol. lvi, pp. 1531-36. Analytical and pharmacological data which could not be incorporated in the preliminary paper are here given.

2. Analysis of the action on the circulatory apparatus.....	364
Action on the vagi.....	364
Action on the blood vessels and other structures containing plain muscle.....	367
The effect on the heart itself.....	370
Diuretic Action.....	374
VI. Summary.....	377

I. HISTORICAL AND GENERAL

Poisons of animal origin, using this term in its widest sense, have always been of interest to the student of medical science, and of late years they have in several instances become of actual value to the medical practitioner. Among poisons of animal origin² may be named the various snake venoms, the poisons of amphibia, of fishes, (e.g., the fugu poison of Japan), of mussels (mytilotoxin), of scorpions, spiders, bees, ants, beetles (e.g., cantharidin), and other insects. Among poisons derived from the organs of mammals may be named those obtained from the thyroid and suprarenal and pituitary glands as having a very high therapeutic value.

While continuing our studies, in the autumn of 1910, on the convulsant action of certain organic dyestuffs,³ acid fuchsin, betanaphthol, phenolsulphonephthalein, and tropaeolin, we had the opportunity of trying the effect of these substances on a tropical toad, *Bufo aqua*. While engaged in this study our interest was aroused by the milky secretion which exudes from its "parotid" glands when the animal is greatly irritated. Scraping off some of the secretion with a knife, we were struck by the bluish-green discoloration which appeared on the blade soon after it had been used. This observation led us to test some of the diluted secretion with ferric chlorid. This reagent was found to develop the characteristic green color of the pyrocatechin reaction. As this reaction is given by the active principle of the suprarenal glands, further tests were at once undertaken with the object of identifying the substance in the toad's secretion which reacts with ferric chlorid as described. It was not a difficult task to demonstrate

² Faust, *Die Thierischen Gifte*. 1906.

³ Barbour and Abel: *This Journ.*, ii, 167, 1910.

that we were here dealing with a substance which is identical with, or closely allied to, the suprarenal principle. Further work on the secretion of the glands demonstrated the presence of a second body which, in respect to its pharmacologic action, is to be classed with substances belonging to the digitalis group of poisons.

The toad has been regarded from the earliest times as a venomous animal. In the Talmud⁴ under the name of *tzab* (from the root meaning to swell or puff up) it is differentiated from the frog and is classed with animals whose touch contaminates. Various peoples have made medicinal use of the toad. The Chinese have long used as a remedy a preparation derived from toadskins which they call *senso*. According to a preliminary notice by Hyashi,⁵ *senso* is an impure product similar in its action to digitalis, but fifty to one hundred times more powerful. Western nations also made use of the toad for medicinal purposes during several centuries and many European medical treatises and pharmacopœias of an earlier day give to the dried toad a prominent place among therapeutic agents. Thus, in the "Thesaurus Pharmacologicus" of Johannes Schröder, published in Leyden in 1672, and in the "Pharmacologia" of Samuel Dale, published in London in 1692, powdered toad is highly recommended for dropsy, bleeding of the nose and other ailments. In the "London Dispensatory" of 1702, edited by William Sa'mon, professor of physick, we read, "They (the toads) are hung up by the neck in the air till they are through dry, and then kept for use. Wierus saith that the poudre of a dry'd toad taken ʒss. at a time or more, cures almost incurable dropsies, carrying away the water by urine. I suppose the ashes of them burned is better." In an abridged text⁶ of some of the medical writings of Michael Etmüller (1644-83), professor of medicine at Leipsic, it is stated that "living toads aroused to

⁴ Hoffmann, D.: Das Buch Leviticus, Berlin, 1905, i, 342. Babylonian Talmud, Kethuboth 15 a; Mishna, Teharoth, v. 1.

⁵ Deutsch. med. Wchnschr., 1911, No. 13, p. 624.

⁶ Michaelis Etmulleri Opera in Compendium redaeta, Londini, 1701, p. 147. In translating these passages we have omitted a few sentences from the paragraph in which they are found as being of less interest to the reader.

the point of fury are venomous, but found dead they are entirely devoid of poison. Transfixed (alive) in the month of July, dried, powdered (the head and entrails being removed) and administered in doses of twelve grains on alternate days they furnish an excellent cure for dropsy. Others administer this remedy in burning fever at its height. Powdered toad is also an effective remedy against incontinence of urine, and is said to be efficacious because of its anodyne character, while its volatile, penetrating salt acts as a diuretic. From it an anodyne oil is also prepared with the aid of sea-salt and sweet almonds."

Toads as remedial agents are further mentioned in the "Pharmacopeia Universalis" of R. James, M.D., London, 1747, in the "English Dispensatory" of John Quincy, M.D., London, 1749, and as late as 1833, but here in a skeptical way only, by J. A. Paris in his "Pharmacologia."

Further testimony that very powerful principles are found in toads is seen in the fact that primitive peoples have made use of their skin secretion as arrow poisons. Thus, Pagenstecher writes:⁷

According to reports made by the botanist André, which have been substantiated by Saffray, the Choco Indians of the primeval forests of the Sierra Templada of New Granada at elevations up to 2000 meters employ for this purpose the secretion of a species of pelobates ("spade-footed" toad). The animal is placed in a tube of bamboo, the hands of the operator being protected with leaves, and when some of the poison is desired the tube containing the toad is suspended high over a fire. The toad soon becomes covered with a yellow juice which is allowed to drop into bowls from which it is transferred to small pots in which it gradually acquires the consistence of curari. A further supply of the poisons may later be obtained from the toads thus treated. The poison is smeared on to the tips of arrows which are shot into game from blowing tubes. A small stag is killed by a poisoned arrow in from two to four minutes, a jaguar in from four to eight minutes.

And in regard to the particular toad which is engaging our attention, Filho⁸ makes the following statements:

⁷ Pagenstecher: *Allgemeine Zoologie*, as cited by Kobert, *Lehrbuch der Intoxikationen*, Edition 2, 1906, ii, 470.

⁸ Filho, Lacerdo, *Algumas Experiencias com o veneno do Bufo ictericus* (Spix), *Archivos do Mus. Nacion. do Rio Janeiro*, 1878, iii, 33.

There exists, more particularly in the regions of the Amazon a species which is a veritable giant among toads and which Spix has described under the name of *Bufo agua*,⁹ whose venom it would be worth while to study. It is very probable that this is the species from which the aborigines of the Amazon derive the poison with which they smear the points of their arrows, in place of a sort of curara which certain other tribes use.

Further details in regard to the use of the poison of *Bufo agua* have not yet come to our knowledge, but our own experiments with it give abundant proof that it would be very deadly indeed when used as an arrow poison.

Bufo agua (*horridus*, *maculiventris*, *marinus*, *humeralis*, *ornatus*, *ictericus*, *Lazarus*, *Rana marina*, *Bombinator horridus*, *Neotes*, *Pseudobufo* and *Docidophryne agua*) is the largest of the *Anura*, or tailless amphibia, attaining a length of 20 or more centimeters and a breadth of 12 cm. It is found in all of the countries and most of the islands of South and Central America, and in the warmer parts of Mexico.⁹

The specimens examined by us were obtained from the neighborhood of Montego Bay, Jamaica, where they are popularly known as bullfrogs.¹⁰ The general color of the animal is light to dark brown with sooty dark patches and its back is covered with warty protuberances. A striking feature is two large oval, so-called "parotid glands" behind each ear. It is from these "glands" that our poison is obtained. According to Seeck,¹¹ these structures consist of simple, elongated glands closely packed together, the whole bearing a resemblance to a honeycomb. When the glands are hardened in alcohol their lumen is filled with a brownish compact mass.

⁹ Brehm, A. C.: Thierleben, Allgemeine Kunde des Thierreichs, 1878, vii, 602; Waite, F. C.: *Bufo Agua* in the Bermudas, Science, 1901, x'ii, 342. Gosse, P. H.: A Naturalist's Sojourn in Jamaica, London, 1851, p. 431.

¹⁰ We are indebted to Prof. E. A. Andrews of this university for much assistance in obtaining a sufficient supply of these animals.

¹¹ Seeck, Oscar: Ueber die Hautdrüsen einiger Amphibien, Diss. Dorpat, 1891. See also a histological study by G. Calmels: Arch. de Physiol., 3d ser., I, p. 321, 1883.

Bristol and Bartelmez, in a short note in *Science*,¹² say of the poison glands of *Bufo aqua*:

The poison glands are found only on the upper surface of the body, while mucous glands are found all over the skin, and are crowded together in large parotoid "glands" behind each ear. They are much larger than the mucous glands and extend deep down into the compact corium layer. They are surrounded by a thin layer of loose connective tissue which contains nerve fibers and a dense net-work of capillaries. There is an almost continuous layer of smooth muscle fibers about the gland. The cells of the glandular epithelium develop to an enormous size, and when they mature they disintegrate, their entire plasm becoming the secretion, so that when a poison gland has reached its full development it is simply a reservoir of poison. When the poison is discharged the remains of the glands are resorbed, and at the same time one of the five or six undeveloped glands, grouped around the mouth of the functioning gland, grows down alongside the remains of the discharged gland, pushing it aside to occupy its former place.

When the animal is irritated as by the bite of a dog or by some other sufficiently powerful stimulus, mechanical, chemical or electric, the parotid glands exude a large amount of a creamy secretion having a pungent aromatic odor. The glands are certainly under the control of the central nervous system, as their secretion is discharged in consequence of a peripheral irritation of sufficient strength. Budgett¹³ states that "the enormous parotid glands are discharged like squirts when the creature is roughly handled." We have not ourselves observed anything of this kind, though we have repeatedly "milked" as many as sixty of the animals at a time. Under chemical and mechanical stimulation we have only noticed free exudation from the openings of the gland. We obtained the needful amounts of this secretion by the simple device of squeezing (or "milking") the parotid glands with a curved hysterectomy forceps, catching the secretion as it spurted out from the numerous orifices into a large glass bowl held inverted over the toad. The semifluid substance thus

¹² Bristol and Bartelmez: *Science*, 1908, xxvii, 455.

¹³ *Quart. Jour. Microsc. Sc.*, 1899, xlii, 305.

obtained quickly dries in the air to form hard, brittle scales of a yellow color, presenting much similarity in appearance to dried snake venom. When the air-dried scales are brought into contact with water they swell up to gelatinous masses, and in the presence of much water an opalescent, neutral foamy emulsion is obtained of a most nauseating bitter taste and pungent odor. In its physical properties, the secretion presents great similarity to some other animal poisons, such as the Habu snake venom described by Ishizaka.¹⁴

Experiments made with the crude poison

A little of the emulsified secretion, well diluted with 0.8 per cent solution of sodium chlorid and instilled into the conjunctival sac of the dog or cat, will quickly cause an extreme constriction of its blood-vessels so that the conjunctiva becomes blanched. This action is due mainly to the epinephrin contained in the secretion, but in small part also to the digitalis-like body. When this latter substance, however, is applied to the eye in the form of a solution of the pure crystals it causes at first a momentary constriction of the smaller vessels of the conjunctiva, but this is soon followed by a marked dilatation, so that great injection and irritation result. No dilatation of the vessels of the eye is observed when only very dilute solutions of the crude poison are used, as in this case the action of the epinephrin preponderates.

A little of the emulsion injected into the abdominal lymph-sac of a pithed frog will soon cause slowing of the heart, with apparent prolongation of the diastolic pause and increase in the ventricular contractions. The final effect is complete stand-still of the heart in systole.

Little, if any, effect is noted when only a small quantity of the dried venom is given to an animal by mouth. Thus, a cat weighing 2.27 kg., received 0.035 gram concealed in meat. Not the slightest objective symptom was noted during the afternoon on which the animal was under observation. In giving the last

¹⁴ Ishizaka: Ztschr. f. exp. Path. u. Therapie, 1907, iv, 88.

piece of meat with the venom concealed in it, however, a minute piece of the poison came into contact with the animal's mouth. The effect was remarkable in causing a profuse flow of foamy saliva. When the animal had ejected the last traces of the poison from its mouth, which was accomplished in a few minutes, it at once returned to its former state of quiet content. The absence of striking symptoms when even a considerable quantity of the crude venom is given by mouth is explicable when it is recalled that the digitalis-like substance of the venom is only slightly soluble in water and that the hard, dry scales of the crude poison require considerable time for their solution. It will be shown later that this principle is highly toxic when injected by itself, either subcutaneously or intravenously, so small a quantity as 1.5 mg. nearly causing the death of a very large cat.

Injected intravenously into a large cat, a quantity of the emulsion containing less than 0.020 gram of the crude dried venom induced a tremendous rise of blood-pressure followed immediately by a fall due to a sudden and complete stand-still of the heart.

When a larger quantity of the dried crude venom is introduced into the stomach more marked symptoms are produced. Thus, 0.1 gram of the poison enclosed in a capsule was given on an empty stomach to a dog weighing 5.8 kg. In ten minutes a clear bile-stained fluid containing most of the poison was ejected. This was followed by retching and repeated vomiting and profuse salivation, all of which continued at frequent intervals for twenty minutes. For an hour following, during which time the animal was kept under observation, it was much depressed. On the following morning it had quite recovered. In regard to this experiment we would say that the amount of epinephrin present in the venom given to this dog would alone suffice to induce vomiting if given on an empty stomach.

It may further be mentioned that an emulsion of the crude venom is a powerful and rapidly acting agglutinating agent¹⁵ for the red corpuscles of the rabbit, the only animal's blood tested in this connection.

¹⁵ Compare Fr. Pröschner on the haemolytic action of an extract obtained from the skin of two European toads, *Bombinator igneus* and *Bufo cinereus*. Beitr. z. chem. Physiol. u. Pathol., I, 575.

II. ON THE PRESENCE OF DIHYDROXY-METHYL-AMINO-ETHYLOL BENZENE, $C_6H_3(OH)_2.CHOH.CH_2NHCH_3$, IN THE SECRETION OF THE POISON GLANDS

Note by the senior author (A.)

Fortune has thrown in our way an animal secretion which most unexpectedly is found to contain a principle which has hitherto appeared only in the suprarenal glands and in homologous chromophil structures. In collaboration with A. C. Crawford, I isolated the active principle of the suprarenal glands of sheep and beeves in the form of physiologically active salts which I named salts of epinephrin, for example, epinephrin bisulphate, picrate, etc. The elementary composition of several compounds was established by analysis and for epinephrin bisulphate, for example, was found to be represented by the formula,¹⁶ $C_{17}H_{15}NO_4.H_2SO_4$.

In all of my published papers¹⁷ of that period (1897-1899) it was constantly emphasized by me that epinephrin is an unstable, basic substance, precipitable from solutions of its active salts by ammonia and capable of being separated from the other constituents of the suprarenal glands by the proper use of benzoyl chlorid.¹⁸ And this at a time, I may perhaps be permitted to say, when von Fürth,¹⁹ another investigator in this field, was upholding the supposition that epinephrin is either tetrahydrodioxypyridin, $C_5H_9NO_2$, or dihydrodioxypyridin, $C_5H_7NO_2$.

Later work²⁰ of mine showed that what I called epinephrin, and salts and compounds of epinephrin, had each and all retained one of the benzoyl radicles which I had purposely introduced into the molecule when I employed benzoyl chlorid as a precipitant. In consequence of the

¹⁶ Abel and Crawford: Bull. Johns Hopkins Hosp., 1897, viii, 151. Abel: Ibid., 1898, ix, 215; Ztschr. f. physiol. Chem. (Hoppe-Seyler's), 1899, xxviii, 318; Bull. Johns Hopkins Hosp., 1901, xii, 339; Ibid., 1902, xiii, 31; Ber. d. deutsch. chem. Gesellsch., 1903, xxxvi, 1839; Ibid., 1904, xxxvii, 368; Am. Jour. Pharmacy, 1903, lxxv, 301; Contributions to Medical Research dedicated to V. C. Vaughan, 1903, pp. 138, 165; Jour. Biological Chemistry, 1905, i, 1.

¹⁷ References are given in my papers to the discovery of Oliver and Schäfer and Szymonowicz and Cybulski, who first noted the presence of a vasoconstrictor principle in the suprarenal glands, as also to the earlier papers dealing with the chemical constituents of the glands.

¹⁸ See Ztschr. f. physiol. Chem. (Hoppe-Seyler's), xxviii, 218.

¹⁹ Von Fürth: Ztschr. f. physiol. Chem. (Hoppe-Seyler's), 1898, xxiv, 142; 1898-1899, xxvi, 15.

²⁰ Abel: Bull. Johns Hopkins Hosp., 1901, xii, 339.

retention of a benzoyl radicle (*an unusual circumstance in work of this kind*) my salts showed a number of so-called alkaloidal reactions in addition to those which belong to the entirely unaltered native compound and which were likewise given by my active salts.

Following this work came the next step, the preparation of an amorphous indigo-colored iron compound of the active principle by von Fürth,²¹ for which, however, at this time no analytic data were given from which a molecular formula could be calculated. Very soon after came the work of Takamine²² and Aldrich²³ who succeeded in precipitating the base from concentrated extracts of the gland by the use of ammonia without the assistance of such agents as benzoyl chlorid or chlorid of iron. The formula $C_9H_{13}NO_3$, first proposed by Aldrich, has proved to be the one which truly represents the composition of our substance, but, as that chemist has remarked, "it is interesting to note in this connection that if we subtract a benzoyl residue from Abel's formula for epinephrin— $C_{17}H_{15}NO_4$ —we obtain a formula— $C_{10}H_{10}NO_3$ —which is not far removed from that of adrenalin." *Such a result is not due to chance and could have been obtained only from the study and analysis of chemical individuals which were fairly pure and which as was later shown had actually retained a single benzoyl radicle, $C_6H_5.CO$.*

Next came the brilliant researches of the chemists, Dakin, Jowett, Pauly, Friedmann, Stolz and Flücher which have finally culminated in the synthetic production, first of the racemic and now of the laevorotatory form as produced in the animal organism itself.

The Council on Pharmacy and Chemistry of the American Medical Association has lately adopted²⁴ the name epinephrin for the active principle of the suprarenal glands in preference to using a "protected" name and it is for this reason that we are using the word in this paper.

As already indicated the fine green color produced by the addition of ferric chlorid to a solution of the crude poison of our toad together with the fact that such a solution finally turns pink when exposed to the air and that it exerts a powerful vasoconstricting action sufficed to show that we had discovered a substance closely allied to, if not identical with the vasoconstrictor principle of the

²¹ Von Fürth: Ztschr. f. physiol. Chem. (Hoppe-Seyler's), 1899-1900, xxix, 105.

²² Takamine: Therap. Gaz., 1901, xxv, 221; Am. Jour. Pharm., 1901, lxxiii, 523.

²³ Aldrich: Am. Jour. Physiol., 1901, v, 457.

²⁴ Proprietary versus Unprotected Names, The Journal A. M. A., March 25, 1911, p. 910.

suprarenal glands. The results of additional tests made with solutions of the crude venom confirmed this conclusion. For example, the addition of ammonia or other alkali brought out a fine pink color which appeared instantly and was intensified by the addition of a trace of iodine or other oxidizing agent, and on boiling with Fehling's solution or with ammoniacal silver nitrate solution reduction of both solutions occurred promptly.

Two methods²⁵ of isolating the newly found substance were employed, only one of which will here be described. From 6 to 15 grams of the air-dried venom were rubbed up with water until a thin, foamy, opalescent solution, or rather emulsion, was obtained. This was repeatedly shaken with fresh quantities of a mixture of ether and chloroform (1 : 4) by which means the digitalis-like body was removed. The solution was then diluted with much water, whereon the mucoid and other contaminating substances were quickly removed by precipitation with basic lead acetate and the pinkish filtrate was *immediately* treated with hydrogen sulphid, freed from the precipitated sulphid of lead and concentrated to a small bulk under diminished pressure. The above operations from the moment of adding the solution of basic lead acetate to that of introducing the sulphuretted hydrogen must be carried on with great celerity, as otherwise there would be danger of injuring the epinephrin, since the filtrate unavoidably becomes slightly alkaline. Then too, the precipitation with basic lead acetate must be undertaken with highly dilute solutions of the venom only, as otherwise too large an amount of epinephrin will be retained in the precipitate of inert substances. When the filtrate, which has been freed from lead sulphid, has been sufficiently concentrated by distillation under diminished pressure it is only necessary to add ammonia to obtain the epinephrin in crystalline form. By dissolving in acetic acid, adding a small amount of sodium sulphite and again precipitating with ammonia, a very pure, ashless and almost colorless product is obtained.

²⁵ The other method was that elaborated by Abel for the isolation of epinephrin from the suprarenal glands of beeves. See Ber. d. deutsch. chem. gesell, xxxvi, p. 1841, 1903.

The results of combustion analyses with products obtained in this way are as follows:

I	II	III
Product Analyzed After Second Pre- cipitation; No Sulphite Used	Product Analyzed After Third Pre- cipitation: Sul- phite Used	Theoretical Require- ments for Formula, $C_9H_{13}NO_3$
C = 58.02	C = 58.68	C = 59.02
H = 6.87	H = 7.56	H = 7.10
N = 7.74; 7.88		N = 7.65

- I. 0.1223 gram substance gave 0.2602 gram CO_2 and 0.0751 H_2O , or 58.02 per cent C and 6.87 per cent H.
 0.2296 gram substance gave 16.05 cc. N at $20.5^\circ C$. under a barometric pressure of 738 mm. from which it is seen $N = 7.74$ per cent.
 0.2082 gram substance gave 14.4 cc. N at $19.5^\circ C$ under a barometric pressure of 758.5 mm. from which it is seen that $N = 7.88$ per cent.
- II. 0.1060 gram substance gave 0.2282 gram CO_2 and 0.0721 gram H_2O , or 58.68 per cent C and 7.56 per cent H.

It will be seen that the analytical results here given, especially those obtained with the more highly purified preparation, stand in good agreement with the theoretical requirements for the accepted formula, $C_9H_{13}NO_3$, and prove that the elementary composition of the epinephrin from the toad is identical with that found in the suprarenal glands of the higher animals.

Like the principle obtained from the suprarenal glands, *Bufo* epinephrin turns the plane of polarized light to the left. Epinephrin (0.078 gram) obtained from our toads and three times crystallized, when dissolved in 8 cc. of water acidulated with acetic acid containing a trace of sodium sulphite, gave a polarimetric reading of -1.00° in a 2 decimeter tube with sodium light, whence:

$$[\alpha]_{\frac{20^\circ}{D}} = 51.30^\circ, \text{ the specific rotation.}$$

This rotation is in perfect agreement with that obtained by Flächer²⁶ (-51.40°) with the highly purified natural product, and differs from that obtained by Abderhalden and Guggenheim²⁷

²⁶ Ztschr. f. physiol. Chem., 1908-1909, lviii, 189.

²⁷ Ztschr. f. physiol. Chem., 1908, lvii, 329.

for the synthetic levo-product (*l*-suprarenin) by the error of the instrument only.

Physiological action

In regard to its physiologic activity—we need only state that the *rapid* injection of so small a quantity as 0.000004 gram (1 cc. of a 0.0004 per cent solution) into the femoral vein of a dog weighing 6 kg. caused a rise of blood-pressure amounting to 20 mm. of mercury as measured in the femoral artery and that the injection of 0.000008 gram (2 cc. of a 0.0004 per cent solution) caused a rise of 26 mm. The physiologic activity of the substance is seen, therefore, to equal that²⁸ of the purest specimens of epinephrin that have been hitherto isolated.

It may also be stated that this epinephrin acts promptly as a vasoconstrictor when applied to the blood-vessels of *Bufo aqua*, the toad from which it is derived. In other words, *the animal has not acquired an immunity against this poison*; small hemorrhages in this toad are as easily controlled by a local application as in other animals, and when a pithed toad is perfused with Locke's solution containing a small amount of the new epinephrin, its arterioles are found to be just as responsive to the drug as are those of the frog (*R. clamata* or *pipiens*).

A protocol of a perfusion experiment (Experiment 1) is here given in which it is shown that 0.0001 gram of bufo-epinephrin very quickly produces a marked constriction of the blood vessels of a pithed toad. It is further to be noted that the vasoconstriction is of considerable duration, the effects of a single injection into the perfusion tube lasting for nineteen minutes. Much smaller quantities of bufo-epinephrin than were used in Experiment 1 were found to be effective, thus so small a quantity as 0.000004 gram injected as above stated was still found to be effective, reducing the outflow from fifteen drops to eight drops in the minute. We have made no quantitative experiments to determine exactly the comparative sensitiveness of *Bufo aqua* and *Rana clamata* to bufo-epinephrin but the above data prove that

²⁸ Schultz, W. H.: Buils. 55 and 61, U. S. Pub. Health and Marine Hosp. Service, Washington, D. C.

this toad is highly sensitive to the action of its vasoconstrictor principle. Fig. 1 gives a part of the tracing in illustration of the protocol of Experiment 1.

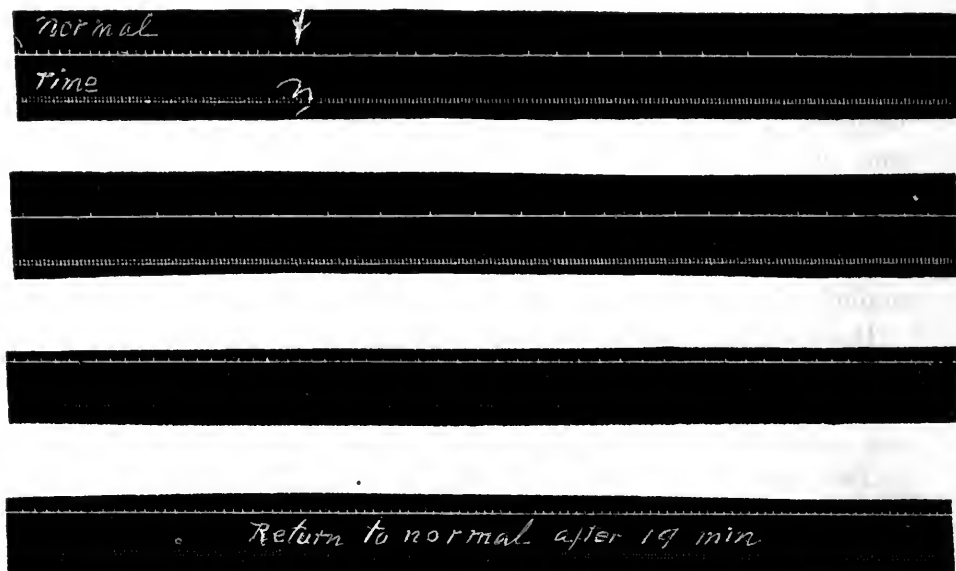


Fig. 1. Perfusion of blood-vessels of *B. aqua* with Bufo-epinephrin at a pressure of 30 cm. Salt solution. Drop Record. Single injection of 1 cc. of a solution containing 0.0001 gm. Bufo-epinephrin injected at ↓ through the rubber tube close to the perfusion cannula in the aorta. Lower tracing in each part of the figure is the time curve (seconds).

Experiment 1. Perfusion of a pithed toad, *B. aqua*, weighing 200 grams with bufo-epinephrin in 0.8 per cent sodium chloride solution under a pressure of 30 cm. The epinephrin solution (1 cc. = 0.0001 gram) was injected into the perfusion tube close to the aortic cannula. Automatic drop record.

Normal number of drops	14 in 30 seconds
30 seconds after injection	4 in 30 seconds
1 minute after injection	3 in 30 seconds
2 minutes after injection	3 in 30 seconds
3 minutes after injection	3 in 30 seconds
4 minutes after injection	4 in 30 seconds
5 minutes after injection	5 in 30 seconds
9 minutes after injection	8 in 30 seconds

11 minutes after injection	9 in 30 seconds
13 minutes after injection	10 in 30 seconds
15 minutes after injection	10 in 30 seconds
17 minutes after injection	11 in 30 seconds
19 minutes after injection	14 in 30 seconds

So, also, *dilatation of the pupil is as easily produced in the excised eye of the toad as in that of the frog.*

It is seen, therefore, that the results obtained by chemical analysis, by the use of the polarimeter, by quantitative and qualitative physiologic experiments have demonstrated conclusively that the substance isolated by us from the poison glands of Bufo aqua is identical with the dihydroxy-methyl-amino-ethylol-benzene, $C_6H_3(OH)_2CHOHCH_2NHCH_3$, produced by the suprarenal glands of the higher animals.

Percentage amount of epinephrin contained in the venom

It is of interest to learn how large an amount of epinephrin may be isolated from a certain weight of the crude venom. Thus, from 5.42 grams of venom, weighed one hour after it was squeezed out of the glands, we obtained 0.243 gram of crystalline epinephrin, a yield of 4.48 per cent. On the assumption that we obtain by this method only about two-thirds of the epinephrin actually present in the crude venom the true content of epinephrin would be nearly 7 per cent (6.72 per cent). Judging from other experiments in which the yield was somewhat smaller, we conclude that the secretion probably varies in its percentage of epinephrin with the season of the year and other circumstances. But as the method of isolation is not, strictly speaking, a quantitative one, exact statements as to the variability of the epinephrin content cannot be made.

If we assume that the venom as secreted by the animal contains from 20 to 30 per cent of water there would still be present, in accordance with the above analysis, about 5 per cent of epinephrin. In comparison with this we would recall that one²⁹ of us estimated

²⁹Abel: Am. Jour. Physiol., 1903, lxxv, 301. See also Reid Hunt: The Comparative Physiologic Activity of Some Commercial Suprarenal Preparations. The Journal A. M. A., Sept. 8, 1906, p. 790.

that the amount found in the suprarenal glands of beeves is 0.3 per cent.

The chromaphil reaction of the poison glands

The presence of this powerful reducing agent in the poison glands of *B. aqua*, makes it almost certain on chemical grounds alone, that their cells will give the histologic reactions of the so-called chromaphil tissue. As is well known, this tissue consists of cell groups which assume a brown color when treated with chromic acid or dichromates in consequence of the reduction of these compounds to brown or reddish brown basic chromates. Such cell groups have hitherto been found only in the medulla of the suprarenal glands, in tissue of similar character lying alongside the abdominal aorta, in the carotid gland and in the sympathetic system.

Mr. E. B. Wood has undertaken the histologic study of the poison glands of *B. aqua*, *B. lentiginosus*, and other toads with especial reference to their chromaphil reactions. His results will be published later but it may here be stated that the poison glands of *B. aqua* (the only toad whose glands have been examined thus far) give the chromaphil reactions with great intensity. Chemical experiments are also in progress in this laboratory for the purpose of learning whether dihydroxy-methyl-amino-ethylol-benzene is produced in the skin glands of other toads and perhaps also in the skin of other amphibia.

III. ON BUFAGIN, A CRYSTALLINE COMPOUND AND METHODS
FOR ITS ISOLATION AND PURIFICATION

It has already been intimated that the venom of *B. aqua* owes its efficacy as an arrow poison in a large degree to the presence of a substance whose pharmacological action is similar to that of digitalis, although the large amount of epinephrin present is an important auxiliary in this action. And it is to the former constituent or to substances closely allied to it, such as have been described by Faust,³⁰ Phisalix and Bertrand³¹ that toadskins owe

³⁰ Arch. f. exp. Pathol. u. Pharmakol., xlvii, 278, 1902; xlix, 1, 1903.

³¹ Compt. rend. de l'Acad. des Sciences, Paris, 135, 47, 1902.

their efficacy as a cure for dropsy, a method of treatment which would perhaps still be in vogue had not William Withering, in 1775, introduced for this very purpose the foxglove, the active constituent of an old wives' remedy. Now that science has begun to study the ancient remedy, toadskin, we may confidently expect further discoveries in this field, with results of undoubted value for practical medicine.

We have isolated the substance here referred to and having obtained it in crystalline form and given proof of its chemical purity and individuality, have named it bufagin in order to use a term sufficiently indicative of its origin.

It is not difficult to isolate bufagin. It is extracted from a solution of the crude venom in water³² by shaking with a mixture of ether and chloroform as already stated and the solvents are removed by distillation until only a small bulk of fluid remains. This is then poured drop by drop with constant stirring into a large volume of petroleum ether which precipitates the bufagin as a white resin while holding the small amount of fatty substances in solution. The resinous precipitate is taken up in chloroform and again precipitated with much petroleum ether, whereon it may be crystallized from absolute alcohol. As bufagin is fairly soluble in this reagent crystallization can only be effected from hot concentrated solutions.

From this point on further purification is effected by recrystallization from alcohol and by repeated crystallization from hot water as will presently be described in detail.

It may here be remarked that in our earlier work we boiled the resinous precipitate (which is obtained by the use of petroleum ether as above described) for several hours with 2 per cent ammonia, in a flask attached to reflux condenser. The object of this treatment was of course the removal of acids or resinous substances of acid character (such as Faust's bufotalin) that might have been carried down in the petroleum ether precipitate. After pouring off the ammoniacal solution the brittle

³² By the use of hot water the solution of the crude venom may be greatly facilitated. In this case the aromatic odor which always accompanies solution is greatly intensified. We have not yet succeeded in identifying the odoriferous body.

resin was dissolved in a very small quantity of chloroform, absolute alcohol containing hydrochloric acid was then added until the mixture contained 1 or 2 per cent of the acid when it was poured into a large quantity of water. In a short time the bufagin had settled to the bottom and sides of the beaker as a resinous precipitate which later changed into masses of crystals. This treatment was necessary in order to remove the last traces of ammonia from the resin. After this treatment the substance was repeatedly crystallized from alcohol and water. As it was found on analysis that nothing was gained by boiling either the petroleum-ether precipitate or the first crystals from alcohol with dilute solutions of ammonia as above described, these steps were later omitted.

The method of isolating bufagin above described in which the crude venom was dissolved in water and then shaken with organic solvents was useful as long as it was desired to recover the epinephrin contained in this portion. When it is not desired to isolate the epinephrin one may proceed as follows: The venom is squeezed from the glands as described, allowed to dry in the air, ground to a fine powder in a closed mill and is then extracted with absolute alcohol until this solvent no longer takes up an appreciable amount of the material. The alcoholic extract is then freed of alcohol and water by distillation under diminished pressure, the residue is repeatedly extracted with chloroform, the chloroform extracts are reduced in bulk by evaporation and then poured drop by drop into a larger volume of petroleum ether. The resinous precipitate which now falls out is again taken up in a small quantity of chloroform and this is again poured into an excess of petroleum ether. The nearly white resinous precipitate now obtained often turns into a crystalline mass in the course of a few days if allowed to stand under the precipitating fluid. The substance is then crystallized twice out of hot alcohol, for which concentrated solutions are required. At this stage the compound has already attained a high degree of purity. For analytical and therapeutic purposes we have, however, proceeded further. The substance is again dissolved in absolute alcohol (1 gram to 80 or 100 cc. alcohol) and this solution is poured drop by drop into a litre of water kept close to the boiling point and well stirred.

The clear aqueous solution is set aside to crystallize. Very soon glittering clusters of whetstone shaped or acicular crystals and prisms begin to appear on the sides and bottom of the containing vessel. A second crop of crystals may be obtained by concentrating the filtrate from the first crop. As thus obtained bufagin may be further purified by a repetition of the above process, or it may again be crystallized from alcohol and then again from water. It will thus be seen that it is not difficult to isolate bufagin or to obtain it in any desired degree of purity.

IV. CHEMICAL PROPERTIES OF BUFAGIN

As thus isolated, bufagin is found to contain no nitrogen and to consist only of the elements, carbon, hydrogen and oxygen. Its melting point³³ is 217–218° C. Although bufagin contains neither water nor alcohol of crystallization its crystals lose their lustre after long standing becoming entirely opaque in consequence of a molecular rearrangement. The substance is easily soluble in chloroform and acetone, fairly soluble in absolute alcohol, but only very little soluble in petroleum ether, carbon disulphide or carbon tetrachlorid and also but little soluble in cold benzene. In glacial acetic acid or in acetic anhydride it dissolves with great ease. It is a “neutral” compound insoluble in aqueous solutions of alkalis or acids. It may be sharply differentiated from cholesterin by the use of the following color reactions:

Color reactions

1. Dissolve a small quantity of dry bufagin in a test tube in chlofororm and stratify under this solution an equal volume of concentrated sulphuric acid. At the line of contact between the two fluids a deep red color will soon develop, but only in the sulphuric acid and when the two fluids are well mixed by shaking and again allowed to separate it will be found that the sulphuric acid has taken up all of the color and is highly fluorescent *while the chloroform remains entirely colorless. Cholesterin* (Sal-

³³ By an error the melting point was given as 188° in our preliminary paper. Jour. Med. Assoc., vol. lvi, 1531, 1911.

kowski's Reaction) *as is well known will cause the chloroform to take on a fine red or pink color* while the sulphuric acid behaves as in the reaction just described. A simple way of showing the difference between the two substances is to dissolve equal quantities in separate test tubes in equal volumes of chloroform, add one drop of concentrated sulphuric acid to each tube and shake well. The tube containing the bufagin remains colorless while that containing the cholesterin at once takes on a carmine red color.

2. Dissolve a small quantity of bufagin in a test tube in fluid trichloroacetic acid and heat on the water bath for a few seconds. A beautiful green color at once develops. Under the same conditions (Hirschsohn's Reaction³⁴) cholesterin gives first a fine pink, then red, then a plum color. Both solutions develop fluorescence on standing for a short time. If the trichloroacetic has been freshly prepared so that but little hydrochloric acid has been evolved, it may be well to add a trace of this acid as otherwise the reaction will not develop so promptly as above described.

3. Dissolve a small quantity of bufagin in a test tube in 1 or 2 cc. of acetic anhydride, then add one drop of concentrated sulphuric acid and shake when a pure green color develops immediately. Cholesterin *in the same quantity* treated in the same manner gives immediately *first* a pink or red color, then a blue or purple and only much later a green color. (Liebermann-Burchard Reaction³⁵). A cholesterin derivative, dihydro-cholesterin (β -cholestanol, Diels) prepared by us according to the method of Willstaetter and Mayer³⁶ gives the above reaction in the same manner as bufagin.

4. Dissolve a small quantity of bufagin in a test tube in $\frac{1}{2}$ cc. of glacial acetic acid add $\frac{1}{2}$ or 1 cc. of acetyl chloride and a few granules of fused zinc chloride and warm very gently for a moment over a small flame. In a few seconds color tints appear which pass rapidly into a fine violet which soon gives place to a rich plum color. This again passes into the end stage of the

³⁴ Pharm. Centralhalle, xliii, 357, cited from W. Glikin in Handb. der Biochemie i, p. 133, 1909.

³⁵ Ber d. deutsch. chem. Gesellsch., xviii, 1804, 1885.

³⁶ Ibid., xli, 2199, 1908.

reaction—a deep green with brownish fluorescence, though it may be necessary to apply a little more heat or to add a very little glacial acetic acid according to circumstances, in order to develop this end stage of the reaction. With cholesterin (Tschugaëff's Reaction³⁷) in place of bufagin a fine cherry red with an eosin-like fluorescence is obtained. The presence of water in the reagents used in this reaction is detrimental to the success of the reaction but much less so with bufagin than with cholesterin. The reaction is one of the most sensitive of the color reactions described and differentiates bufagin sharply from cholesterin.

5. As might be expected, the reaction of Denigès³⁸ which is a combination of the reactions of Salkowski and Liebermann gives a negative result with bufagin. As described under 1, so here too with bufagin the chloroform layer remains entirely colorless, while with cholesterin it assumes a brilliant-red color.

Other reactions were not tried, but the above suffice to show how *differently* bufagin behaves from cholesterin when treated as described.

Bufagin differs from cholesterin not only in its response to color reactions, as shown above, but also in its influence on the plane of polarized light, being dextrorotatory while cholesterin is laevorotatory.

A specimen of Product III, the analytic data for which are given below, weighing 0.160 gram, dissolved in 8 cc. of chloroform gave a polarimetric reading of $+ 0.44^\circ$ in a 2 decimeter tube with sodium light from which it is seen that the specific rotatory power of

the compound is $[\alpha]_D^{24} = + 11^\circ$

Analyses

Combustion analyses show that the elementary composition of bufagin can only be expressed in the formula, $C_9H_{12}O_2$, or in terms of a multiple of this formula, such as $C_{18}H_{24}O_4$. The following tables give some of our analytical results, with specimens dried over sulphuric acid and not heated.

³⁷ Zt. f. angew. Chem., Nr. 25, 1900. Cited from Hdb. d. Bioch. I. 133.

³⁸ See, W. Glikin in Oppenheimer's Hdb. d. Biochemie, i, p. 133.

I	II
Obtained directly from venom, but crystallized from dilute solution in petrol ether + chloroform without any other process of purification.	Product obtained by crystallization from alcohol after a preliminary process of purification.
C = 70.84	C = 70.98
H = 8.67	H = 8.55
III	IV
Product same as in II, only was further purified by crystallization from water.	Theoretical requirements for the formula, $C_9H_{12}O_2$ or for $C_{18}H_{24}O_4 = (C_9H_{12}O_2)_2$
C = 71.07 70.92 H = 8.28 8.25	C = 71.01 H = 7.95

It will be seen that our analytical results, especially those obtained with the pure Product III are in perfect agreement with the elementary formula, $C_9H_{12}O_2$ or with some multiple of it as $C_{18}H_{24}O_4$. This Product III was twice crystallized from absolute alcohol and twice from hot water in addition to having undergone the preliminary precipitations with petroleum ether for the removal of fats and cholesterin and also the rather needless treatment with ammonia previously described.

- I. 0.0891 gram substance taken gave 0.2311 gram CO_2 and 0.0695 gram H_2O , or 70.74 per cent C. and 8.67 per cent H.
- II. 0.1021 gram substance taken gave 0.2668 gram CO_2 and 0.0790 gram H_2O , or 70.98 per cent C and 8.55 per cent H.
- III. 0.1812 gram substance taken gave 0.4722 gram CO_2 and 0.1351 gram H_2O , or 71.07 per cent C and 8.28 per cent H.
0.1694 gram substance gave 0.4405 gram CO_2 and 0.1257 gram H_2O , or 70.92 per cent C and 8.25 per cent H.

The material used in the above analyses was dried at room temperature *in vacuo* over sulphuric acid. It appears not to contain either water of crystallization or alcohol of crystallization and can not be dried at high temperatures without undergoing change. When the compound which has been dried to constancy of weight *in vacuo* at room temperature is heated for one hour at a temperature varying from 100–115° C. in a current of dry hydrogen it will lose 4.24 to 4.32 per cent of its weight³⁹

³⁹ The material used in these experiments had been crystallized from alcohol only and had a slightly larger content of hydrogen than is demanded by the formula $C_{18}H_{24}O_4$. It is possible that material crystallized from water would not show this loss.

in this time. In one experiment of this kind an attempt was made to collect the products given off to the current of hydrogen. The drying temperature was maintained at 96–99° C. for an hour except for a few moments when it reached 107°. The products given off were passed through a mixture of solid CO₂ and ether whose temperature was about –80° C. 0.1388 gram substance lost 0.006 gram in the hour, or 4.32 per cent, but of this amount only 0.0016 gram (1.15 per cent) was retained by the cooling mixture. In other experiments carbon dioxide was identified as one of the products given off. It is apparent therefore that bufagin undergoes a slight decomposition when heated to 100° C. or higher in a current of hydrogen.

Behavior of Bufagin toward Bromine

The behavior of bufagin toward bromine is different from that shown by cholesterin, which compound, by virtue of its unsatisfied affinities, readily absorbs bromine, forming with it a colorless crystalline di-bromide. If 50 mgs. of bufagin be dissolved in 1 or 2 cc. of chloroform or carbon disulphide and a solution of bromine in one of these solvents added, not the least diminution in the intensity of the color is to be noted. After standing for an hour, especially if an excess of bromine is present, a part of the bufagin may fall out in the form of a dark brown resin. This resin, which holds bromine in combination, is apparently a substitution product, but we have not yet been able to obtain it in crystalline form. That hydrobromic acid is produced in the reaction above described is easily demonstrated, and we may therefore conclude that bufagin does not contain *an unsaturated carbon linkage* and that bromine acts upon it only to form a brown substitution product of resinous character.

*Behavior toward Platinum-black and Hydrogen*⁴⁰

Eleven one-hundredths gram of bufagin was dissolved in much ether, about 2 grams of freshly prepared platinum-black were added and a slow current of hydrogen was passed through the ethereal solution for forty-eight hours. The resulting product

⁴⁰ See Willstaeter u. Mayer, Ber. d. deutsch. chem. Gesellsch., xli, 2199, 1908.

was a white resin which differed markedly from bufagin in that it could no longer be crystallized from hot water; it now behaved more like cholesterin than bufagin, appearing in water as a colloidal suspension. Its behavior toward such of the color reactions as were tried was also different from that of bufagin. Thus, Hirschsohn's reagent and also acetic anhydride and concentrated sulphuric acid now gave the same play of colors as appear when cholesterin is treated with these reagents. We think it very likely that oxygen was removed in the course of the long reduction and that a substance was thus formed whose properties are more like those of cholesterin than of bufagin. This reduced bufagin was also found to be non-toxic, 0.012 gm. given subcutaneously having no observable effect on a dog weighing 4 kg.

Reaction with Phosphorus-pentachloride

Bufagin reacts as easily with phosphorus-pentachloride as does cholesterin and yields what is apparently a crystalline product corresponding to cholesteryl-chloride and which may be called bufagyl-chloride. A description and analyses of this and other derivatives which we hope to prepare must be reserved for a future paper.

Determination of the molecular weight

Since the publication of our preliminary paper molecular weight determinations of bufagin have been very kindly made for us by Professor Harry Jones of this University and by Dr. George Barger of the Wellcome Research Laboratories. Professor Jones' determination was made by the boiling point method with chloroform as the solvent and the data and results obtained are as follows: 1.5368 grams of bufagin crystallized from water (melting point, 218° C.) raised the boiling point of 90.338 grams of chloroform 0.212° C., from which the molecular weight is calculated to be 294. The molecular weight called for by the formula $C_{18}H_{24}O_4$, which, as has been shown, expresses the results of our elementary analyses, is 304.

Dr. Barger's determinations were made by his microscopical method.⁴¹ A determination in chloroform gave a molecular

⁴¹ Trans. Chem. Soc., lxxxviii, 286, 1904.

weight of 382. Inasmuch as substances which contain one or more hydroxyl groups are associated in this solvent, especially when it is used at room temperature this high figure is explainable. With pyridine in which solvent association never occurs, according to Barger, the determination gave a molecular weight of 288. The data for this determination are as follows: 0.0120 grams bufagin was dissolved in 0.49 gram pyridine, equivalent to a molar concentration of 0.08 to 0.09. The determination gave the molecular weight 288, a result which stands in good agreement with the theoretical requirement 304.

We have here the determinations of two independent observers, made by different methods and with different solvents, which are in close agreement with each other and from which we may conclude that the formula of bufagin is $C_{18}H_{24}O_4$.

Possible relationship to Cholesterin

One is tempted to assume that bufagin is intimately related to cholesterin when one recalls the behavior of the substance toward the cholesterin color reactions as already described. It must be borne in mind, however, that a number of resin acids, terpenes, and unsaturated alcohols of high molecular weight give very similar color reactions, and in this connection it may be stated that cholesterin is declared by Windaus (Arch. der Pharmacie, cccxvi, 147, 1908) to be without doubt itself a complex terpene. No known derivative of cholesterin has the formula of bufagin, $C_{18}H_{24}O_4$, and none of the numerous oxidation products of cholesterin which have been studied exhibit the pharmacological properties of bufagin. For the present, therefore, no definite statements can be made in regard to the relationship of bufagin and cholesterin.

Faust⁴² and his pupil Flury⁴³ have shown that a number of acidulous oxidation-products of cholesterin, $C_{27}H_{44}O_4$, $C_{27}H_{40}O_5$, and $C_{27}H_{40}O_8$ which were prepared by Windaus are pharmacologically active substances, but the behavior of these acids is such

⁴² See works of Faust already cited and monograph, "Ueber das Crotalotoxin," etc., Leipzig, 1911.

⁴³ Arch. f. Exp. Pathol. u. Pharmacol., lxvi, 221, 1911.

that they must be classed with the biliary acids and the saponines, rather than with bufagin.

Lifschütz⁴⁴ maintains that the blood and certain other organs of higher animals contain oxycholesterins, substances which represent the first stages in the physiological oxidation of cholesterol. The liver contains only mere traces of these oxycholesterins and this author assumes that they are changed into bile acids in this organ. The evidence for the existence of these oxycholesterins of Lifschütz in the animal economy rests entirely on spectroscopic observations made upon certain extracts treated with glacial acetic and concentrated sulphuric acids. We may well grant the existence of oxycholesterins in the animal organism *but it should be noted that they have not yet been isolated as chemical individuals of even approximate purity*,⁴⁵ and that we are entirely ignorant in regard to their physiological significance.

A brief consideration of the earlier chemical investigations on toad venom

As far as we have been able to learn, ours is the first chemical study to be made of the venom of *Bufo aqua*; during the last fifty years, however, repeated attempts have been made to isolate the active principle or principles of the skin secretion of *Bufo vulgaris* and *Bufo viridis*, species widely distributed in Europe. Some account of these earlier investigations will be found in the comprehensive monograph on Animal Poisons of E. S. Faust. This author, Phisalix and Bertrand⁴⁶ are the only investigators known to us who have made chemical studies in the past decade of the venom of *Bufo vulgaris*, as obtained either from the whole skin (Faust) or from the secretion of the parotid glands (Phisalix and Bertrand).

According to Faust⁴⁷ the chief poison of the common toad is an amorphous, resinous substance—of acidulous character, readily soluble in aqueous solutions of the alkalies, and endorsed with the

⁴⁴ Zeitschr. f. physiol. Chem., lviii, 175, 1908; lxiii, 222, 1909. Ber. d. deutsch. chem. Gesellsch., xlv, 252, 1908.

⁴⁵ See criticism by A. Windaus, Arch. der Pharmacie, cxxlvi, 148, 1908.

⁴⁶ Compt. rend de l'Acad. des Sc., Paris, cxxxv, 46, 1902.

⁴⁷ Archiv. f. Exp. Pathol. u. Pharmacol., xlvii, 278, 1902; and xlix 1, 1903.

pharmacological activity of the digitalis group. This resin was named by Faust bufotalin and to it he ascribes the molecular formula $C_{34}H_{46}O_{10}$.

He describes a second substance, pharmacologically similar to bufotalin but weaker in its action, as crystalline and having the formula $C_{34}H_{54}O_2$. He names this second substance bufonin and suggests that it is the mother substance of bufotalin which is derived from it in the body of the toad by oxidative processes. Both substances are thought by him to be derivatives of cholesterolin.

Phisalix and Bertrand, who used the expressed contents of the parotid glands, declare that the venom contains but one poison of the digitalis type, not two as maintained by Faust, and they find that it is a noncrystalline resin of neutral properties, not acidulous as is bufotalin. They accepted Faust's name bufotalin for their neutral resin, contending, however, that Faust's form of it was contaminated with substances of acid character derived from the skin of the toad. To their bufotalin they ascribed the formula $C_{119}H_{117}O_{25}$.

As to Faust's second substance bufonin, Bertrand⁴⁸ maintains that it is ordinary cholesterolin slightly contaminated with the neutral bufotalin of Phisalix and Bertrand, a contamination sufficient to give it a slight degree of digitalis-like action. These authors also assumed on physiological grounds the presence of a second poison which they define as a paralyzant of the central nervous system. They did not isolate this assumed poison but named it bufoténine. Faust on repeating their work was unable to obtain a substance of this character.

It will therefore be seen that the true nature of the digitalis-like principle of the common-toad of Europe is a matter of controversy. According to Faust it is a resin of acidulous properties according to Phisalix and Bertrand a neutral resin.

In view of our isolation of the crystalline compound bufagin and the possible relationships suggested by the work of the authors above cited, a reëxamination of the venom of *Bufo vulgaris* and *Bufo viridis* would be highly desirable.

⁴⁸ Compt. rend. de l'Acad. des Sc., cxxxv, 49, 102.

V. PHARMACOLOGICAL ACTION OF BUFAGIN

It is not our purpose to give a complete account of the numerous pharmacological studies that have been made up to the present time with the venom of the common toad of Europe or with crude extracts and active principles of doubtful purity derived from it. In several particulars the pharmacological investigations of the earlier writers have not been improved upon to this day, as may be seen for example, by reading Fornara's (1877) account of the action of the venom upon the exposed heart in various species of animals. A more complete analysis of the pharmacological action of the venom is given only by investigators of recent date.

The pharmacological studies of E. S. Faust⁴⁹ were made with his bufotalin and bufonin and are concerned chiefly with the action of these substances upon the circulatory apparatus. It was shown that these principles act like bodies of the digitalis series and in this respect they reproduce the action of the crude venom and agree with the action of the principle obtained by us from the tropical toad, *Bufo aqua*.

From the pharmacological point of view the investigations of the Russian pharmacologist N. P. Kravkov⁵⁰ are also of importance.

This investigator made use of an alcoholic extract of the expressed contents of the parotoid glands of *Bufo Viridis* and *Bufo Vulgaris* and his material, therefore, would be much like the crude resin (bufotaline) of Phisalix and Bertrand, and therefore can not claim to represent a single chemical individual. This work is nevertheless interesting and important, and we find that as far as it goes it stands in close agreement with the pharmacological effects noted by us in the use of pure bufagin. Thus, the fatal dose of his poison for a dog, 0.7 mg. per kilo of body weight is practically the same as that of our drug; and his experiments on the action of the venom on the blood pressure of intact and decerebrate dogs, on the isolated kidney and on diuresis all reveal in general the same physiological action as is observed with bufagin.

⁴⁹ Arch. f. Exp. Pathol. u. Pharmacol., xlvii, 278, 1902.

⁵⁰ Russki. Vrach, iii, 761, 1904.

A. *Action of Bufagin on cold-blooded animals.*

a. *Experiments on the frog and terrapin.* Doses of bufagin from varying 0.1 to 0.3 mg. injected into small frogs (*R. clamata*) varying in weight from 20 to 30 grams, produce few if any symptoms. If a dose of 0.5 mg. is injected there is at first no effect. In about an hour after the injection, however, the frog seems to have partially lost its reflexes and moves about as if its legs were stiff. This condition lasts an hour or two, gradually wearing off in this time. The minimum lethal dose for a frog is about 1 mg. per 20 grams of body weight and the effect of such a dose is illustrated by the following protocol.

Experiment 2. Frog, *Rana clamata*, 20 grams.

1.40 p.m. Injected 1 mg. of Bufagin in 2 cc. alcoholic solution (5 per cent alcohol). An equal amount of an alcoholic solution of the same strength was injected under the skin of a control frog.

2.00 p.m. Frog a little stiff in its legs.

2.15 Frog partially paralyzed, lies with its belly touching the table; cannot jump; when placed on its back turns over with great difficulty. Reflexes have disappeared.

2.30 Rests completely flat on table, cannot move or turn over when placed on its back. Slight twitching of abdominal muscles.

The frog is now pithed, its brain and spinal cord destroyed and abdomen opened, the abdominal veins are seen engorged with blood. The heart on being exposed shows the ventricle to be pale, it does not expand much in diastole and the auricles are much dilated and engorged with blood. Heart rate fourteen per minute.

2.40 Pericardium opened, ventricle pale; beats are slow and powerful, twelve a minute.

2.45 Heart beat eight a minute.

4.00 Heart again assumes normal rate, ventricle dilates and becomes well-filled during diastole, heart is recovering. A control experiment made with an equivalent quantity of alcohol gave no such effect.

In the above experiment we see the characteristic action of the drug, namely, a gradual slowing of the heart with an increase of its tonicity and in the force of its beat. In the above case, as far as can be judged by inspection, this increase in tonicity did not go

as far as a final arrest in systole, but a slightly larger dose would have produced that effect.

The same action of the drug may be observed on pithing the frog, opening its pericardium and exposing the heart, and introducing at the same time a few drops of a solution of bufagin into the opened pericardial sac. The action on the excised frog's heart is further illustrated graphically by a suspension experiment (Fig. 2). The tracing shows a marked increase in tonicity, gradual slowing of the beat, and final standstill with systolic contraction.

The effect of bufagin on the terrapin's heart, as far as studied, is exactly the same as on the frog.

We have also studied the action of the drug on the frog's blood-vessels by means of perfusion experiments and find that it has a decided action on the plain muscle of the arteries. Aerated Locke's solution under a pressure of 30 cm. was used as the perfusing fluid and at a given time a small quantity of fluid (2 cc.) containing 0.00044 gram bufagin was injected through the rubber tube close to the perfusing tube. Before the injection the number of drops of outflow from the cannula in the *sinus venosus* was nine in twenty seconds. Twenty seconds after the injection it had fallen to six in twenty seconds, one minute later it was four drops and five minutes later three drops in twenty seconds. This marked vaso-constrictor effect continued undiminished for ten minutes longer.

b. Experiments on the toad, Bufo aqua. That the common toad of Europe is relatively immune to its own venom as well as to the various members of the digitalis series of cardiac poisons has been known since the time of Vulpian⁵¹ and has been confirmed by more recent writers.

Fühner,⁵² although admitting the high resistance of the common

⁵¹ Vulpian: Compt. rend. Soc. de Biol. 2nd Series 1854, i, 133; 2nd Series 1856, iii, 125; Fornara; Rivista clinica di Bologna, 2nd Series, iii, 299, 1873; Kobert, Arch. f. Exp. Path. u. Pharmakol, xxii, 104, 1887; Honda: Arch. internat. de Pharmacodyn. et de Thérapie, ix, 431. 1901; Heuser: ibid., x, 483, 1902.

⁵² Arch. f. exp. Pathol. exp. u. Pharmakol, lxiii, 374, 1910.

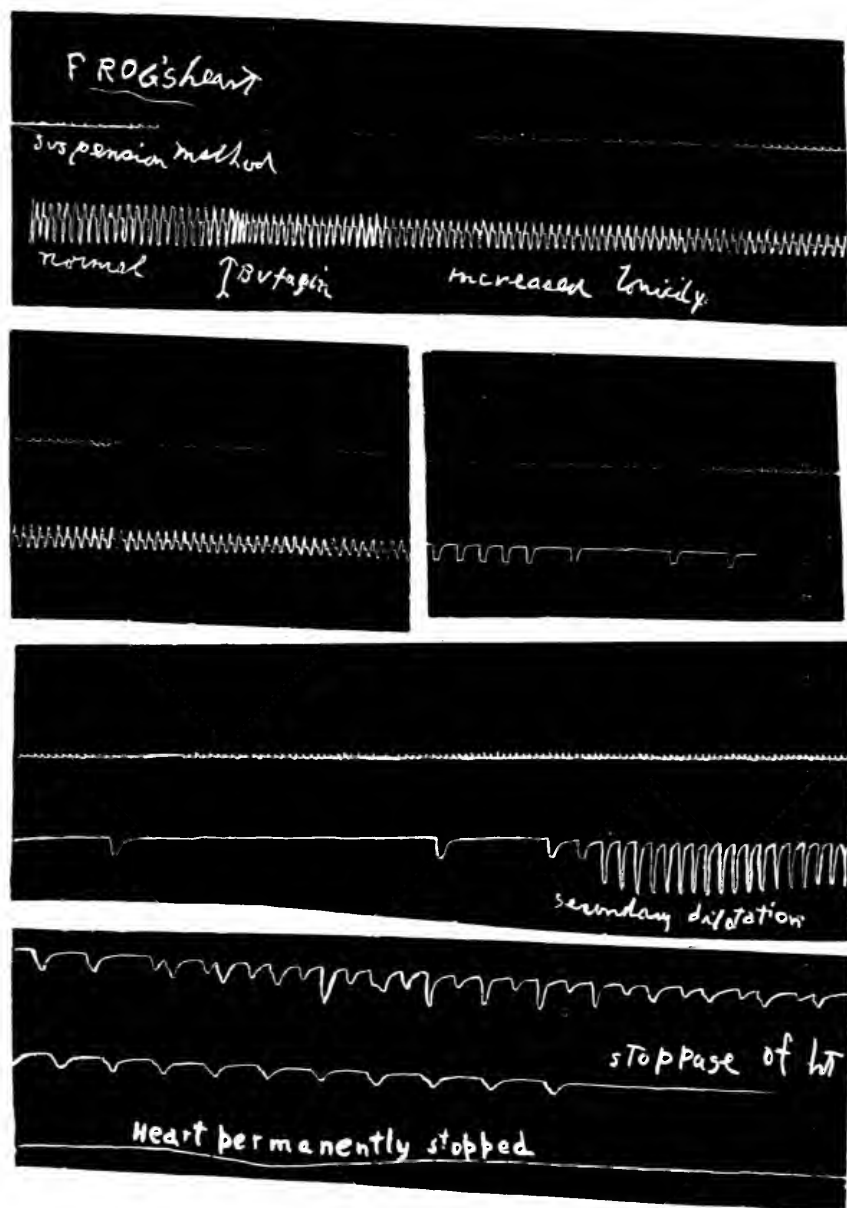


Fig. 2. Action of bufagin on excised frog's heart. Suspension method. Aerated Locke's solution, (at \uparrow) replaced by Locke's solution, 25 cc., containing about 0.002 gm. bufagin. Upstroke = systole, upper tracing = time in seconds, except in lowermost part of the figure in which the time curve is lacking.

toad to the subcutaneous or stomachic administration of its own venom (Vulpian), concludes from his own experiments made with an aqueous extract of the entire skin of the toad that the heart of this animal is by no means immune to the venom. The extract used by Fühner, as is especially pointed out by him *very quickly* induced standstill and systolic contracture of the heart of the frog and of the toad, though the contracture was somewhat less developed in the toad than in the frog. In the use of an aqueous extract such as was used by this investigator various constituents of unknown activity may obscure the effects of the true systolic heart poison. As will be seen from our experiments with bufagin the pure drug does not cause an immediate systolic arrest of the heart of the toad but only slowly alters its rhythm, tonicity and conductivity and finally leads to a perfectly developed systolic contracture.

Our experiments have shown that while *Bufo aqua* is not in the least immune to the epinephrin contained in its venom, its resistance toward bufagin is greater than that of the frog (*R. clamata*). That this toad is only relatively immune to this systolic heart poison contained in its venom may be seen from the protocols of the following experiments. An injection of 1.5 mg. has no observable effect but an injection of 8 mg. has a depressant action from which the animal soon recovers.

Effect of Bufagin on Bufo Aqua

Experiment 3. May 3, 1911 *Bufo Aqua* 100 grams injected intraperitoneally with 2.5 cc. of solution of Bufagin (= 1.5 mg). No effect noted.

May 4 Alive and well; but seems a little sluggish in its movements.

May 7 Alive and normal.

Experiment 4. December 4, 1911, 2.30 p.m. *Bufo Aqua* 225 grams. Injected intraperitoneally with 10 cc. = (8 mg. bufagin) of 5 per cent alcohol solution.

2.40 Is partially paralyzed; lies with its belly touching the table.

3.45 Stretches legs out more sluggishly than usual.

4.00 Animal apparently normal. All that can be noted is a little

slowness in movements. Otherwise, it responds to stimuli, turns over when placed on back, and hops as usual. Skin is very *moist*, much more so than at the beginning of the experiment.

December 5 Animal has recovered and is in normal condition. A control experiment was made with an equivalent amount of 5 per cent alcohol. The toad was slightly depressed for half an hour after injection, but soon recovered and was in normal condition.

Experiment 5. March 21, 1911 *Bufo Agua* 120 grams, pithed, chest opened and heart exposed. Rate of heart-beat, fifteen in fifteen seconds.

12.30 Applied to heart 0.1 cc. of bufagin solution (1 cc. = 0.75 mg.).

One minute later rate of heart-beat, six in fifteen seconds; five minutes later rate of heart-beat, ten in fifteen seconds.

2.30 Injected into aorta 0.3 cc. of bufagin solution (1 cc. = 0.75 mg.). Rate becomes eight in fifteen seconds; contractions of heart are markedly more powerful.

2.45 Injected into aorta 1 cc. of same solution.

2.50 Rate is eight in fifteen seconds.

3.30 No change.

As far as the action of bufagin on the heart of *Bufo aqua* is concerned, our experiments show that the direct application of quantities varying from 0.075 to 0.25 mg. cause a distinct slowing of the heart beat which is, however, of short duration only. When the action of the drug on the excised heart of the toad is studied by the suspension method (ventricle) it is seen that the tonicity of the heart is greatly increased and its rate of beat lessened.

Finally a most interesting phenomenon was observed which was never seen in the frog. Periods of absolute inhibition of the ventricle alternate with periods of action (see Fig. 3). These alternating periods of inhibition and activity of the ventricular rhythm are approximately of equal duration. During the periods of inhibition of the ventricle, the auricles are seen to beat ineffectively and slowly and it is for this reason that the condition may be described as one of heart block due to lack of transmission of impulses in the bundle of His. Studies of the ventricular output of the heart of the toad and frog were not undertaken, as it was planned to make such studies on the warm-blooded heart,

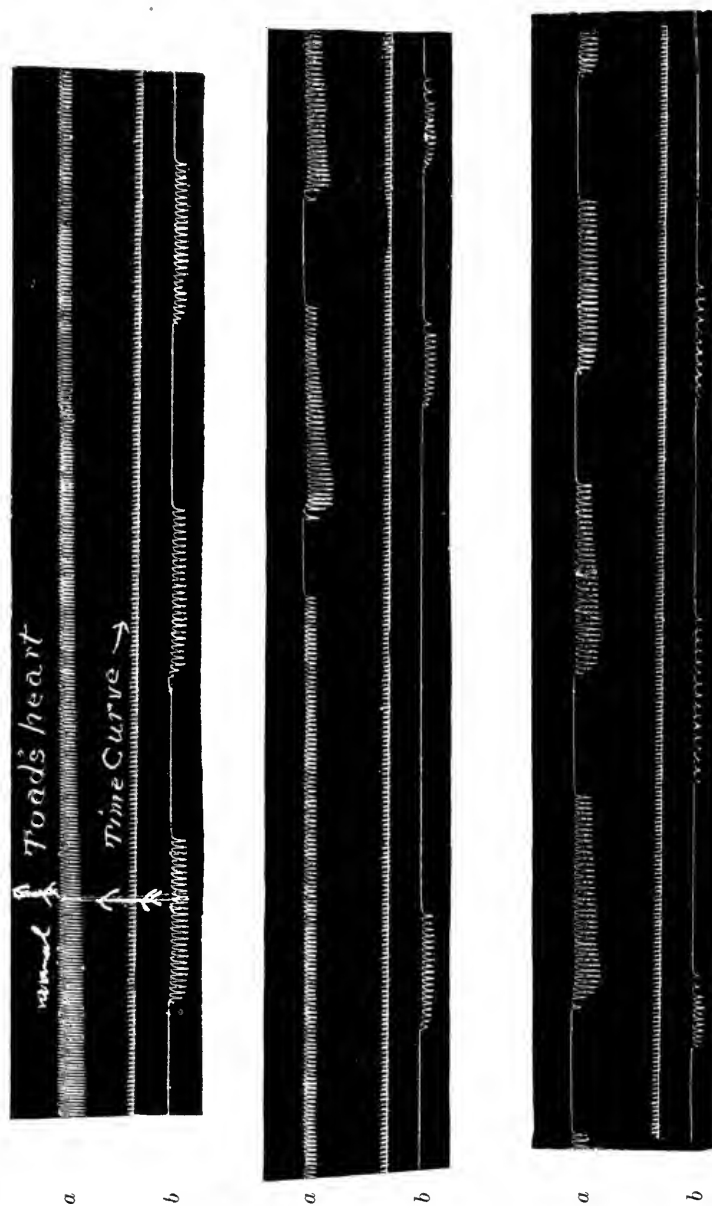


Fig. 3. Action of bufagin on excised heart of *Bufo agui*. Suspension method. Upstroke = systole. Aerated Locke's solution replaced at ↑ by Locke's solution containing 0.002 gram bufagin.

The action of bufagin on the walls of the blood vessels of *B. aqua* does not seem to differ in any way from its action on those of the frog, unless it be that the effect of small doses is more marked in the latter. On perfusing the toad with a solution of bufagin, a marked vaso-constrictor effect is observed, as may be seen by a study of the protocol of Experiment 6. Fig. 4 contains a portion of the graphic record of this experiment. The drug exerts a similar action on the plain muscle fibers of the stomach of *B. aqua* causing them to contract (Fig. 5).

Experiment 6. January 30 and 31, 1911 Perfusion of *Bufo aqua* with bufagin. Perfusing cannula in aorta, outflow cannula in sinus venosus. Locke's solution perfused under a pressure of 35 cm. At stated times solution of bufagin is injected through the wall of the rubber tubing close to the aortic cannula.

January 30, 1910

TIME	NUMBER OF DROPS IN TWENTY SECONDS	REMARKS
3.40-3.50.....	24	{ Injected 1.5 cc. = 0.00033 gram bufagin. Effect is immediate; fifteen drops in first twenty sec- onds.
3.50, injection.....		
3.51-4.01.....	9	{ Injected 1.5 cc. = 0.00033 gram bufagin; immediate effect as be- fore, eight drops in first twenty seconds.
4.01-4.15.....	10	
4.15-4.30.....	10	
4.30-4.35.....	10	
4.35, second injection ...		
4.36-4.45.....	9	{ Toad placed on ice until the morn- ing of the 31st, when the perfu- sion was continued as before, first with normal Locke's solution.
4.45-4.53.....	9	
January 31st.....		
10-10.36.....	13	1.5 cc. = 0.00033 gram bufagin.
10.37 first injection.....		
10.37-10.48.....	9	1.5 cc. = 0.00033 gram bufagin.
10.48-10.53.....	9	
10.53 second injection...		
10.53-10.55.....	8	
10.55-11.00.....	7	
11.00-11.03.....	6	

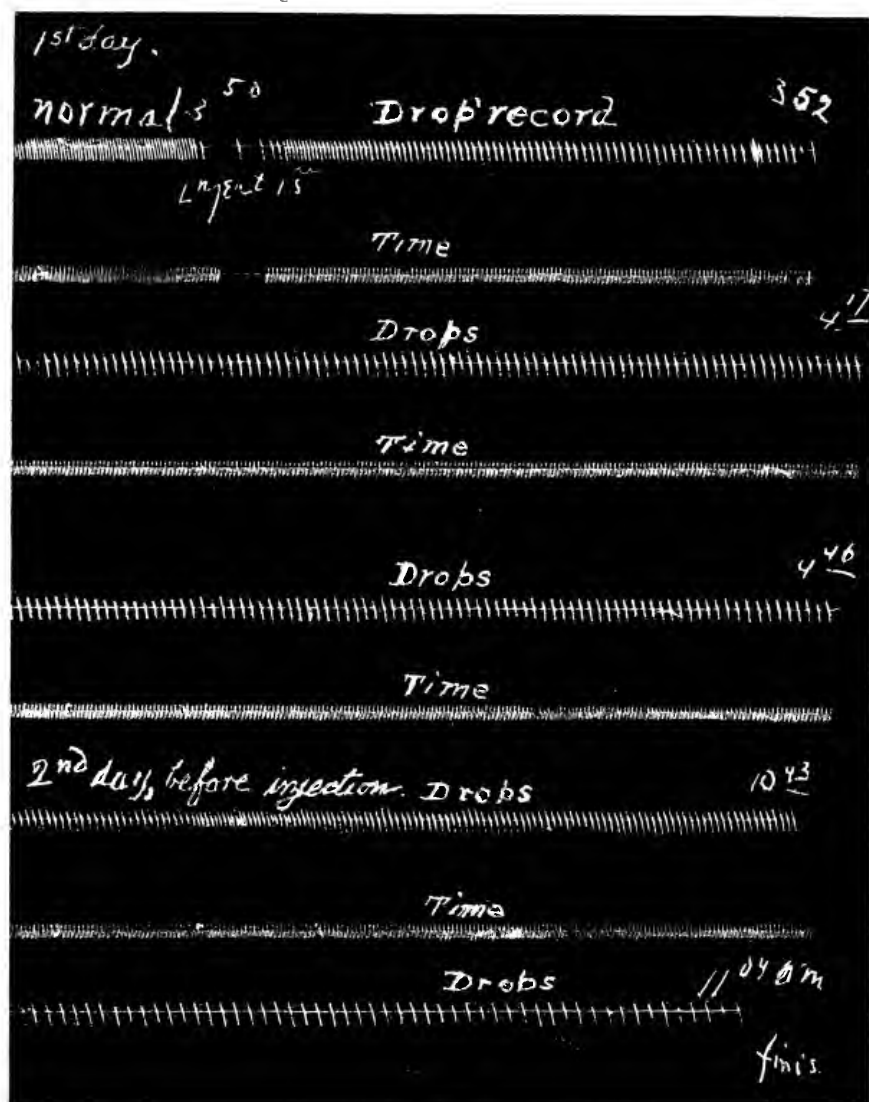


Fig. 4. Perfusion of *B. aqua* with bufagin. Locke's solution; perfusion pressure = 35 cm. At intervals as stated in protocol 0.00033 gram bufagin was injected into the perfusion tube.



Fig. 5. Excised stomach of *B. aqua*. Suspended in 30 cc. 0.8 per cent aerated salt solution. Piston recorder. At ↑ solution was replaced by one containing 0.2 mg. bufagin.

B. Action of Bufagin on Warm-blooded Animals (Mammalia)

Effects on the intact animal. Our observations were made on white mice, guinea-pigs, rabbits, cats and dogs.

The drug was administered hypodermically, intramuscularly, and by mouth. We have also made some observations on the effect of the drug on man.

We have made experiments with a view of detecting any local irritation or infiltration following hypodermic administration of the drug, and have convinced ourselves that bufagin in the doses used causes no local irritation whatever, unlike digitoxin and other members of the digitalis group of drugs.

As bufagin is but very sparingly soluble in water or in physiological salt solution, (0.2 mg. to 1 cc. water at room temperature), and tends to crystallize out from aqueous solutions containing even this small amount, the solutions in most of our experiments were made up with small quantities of alcohol, the amount of which varied from a fraction of 1 per cent to 5 per cent. Control experiments with equal quantities of alcohol were made, and we have found that the transient local symptoms following injections of bufagin were no greater than those following injections of solutions containing alcohol (1 per cent to 5 per cent) only.

As a result of our observations it was found that the herbivorous animals studied, the guinea-pig and rabbit, are relatively more resistant to the action of bufagin than the carnivorous animals, the cat and dog, the minimal fatal dose for the guinea-pig being over 2.5 mg. and for the rabbit 4.6 mg. per kilo, whereas for the cat it was 1.8 mg. and for the dog 0.76 mg. per kilo.

Special observations were made by us to ascertain the existence of any *cumulative* action following the administration of bufagin. Thus, a rabbit weighing 1200 grams received ten injections of bufagin, varying in strength from 0.25 mg. to 0.75 mg. in the course of *five days*, and was kept under observation for several weeks afterwards. In another case a young dog weighing 3.9 kilos, received daily injections of 1 mg. over a period of two weeks. *In no case was there any evidence of a cumulative effect noted.*

We have numerous protocols of experiments illustrating the

effect of bufagin on the intact animal, but it would take too much space to present them all here.

We will therefore give only a few illustrative examples, and content ourselves with summarizing briefly the general effect of the drug on all of the animals studied.

The systemic effects following administration of *small* doses of bufagin are manifested by the slowing of the pulse-rate, this being sometimes preceded by a short period of acceleration, and by a strengthening of the heart beat, as judged by the stethoscope and by palpation. The respiratory movements show very little change in the first stage of the action of the drug unless there is nausea or vomiting. Later these movements may be somewhat more vigorous and in the last or toxic stage they may be very deep and powerful and of a markedly abdominal type.

The toxic effect of larger doses is manifested in an extreme slowing and irregularity of the heart beat, vomiting and salivation, involuntary passing of urine and feces, incoördination of movements, and finally clonic convulsions which may possibly be due to the insufficiency of the circulation in the last stage. On autopsy the heart in almost all cases is found contracted in systole.

Illustrative protocols

Experiment 7. A white mouse, weighing 12 grams was injected intraperitoneally with 0.25 cc. of bufagin solution (1 cc. = 0.75 mg.). One minute after injection animal falls over in violent convulsions, with very rapid respiration, marked salivation and involuntary flow of urine and feces. Convulsions last two minutes and end in death.

It was found that a much smaller dose 0.05 mg. could induce convulsions in another mouse 12 grams, which were followed by recovery.

Experiment 8. May 30, 1911 Guinea pig 200 grams.

11.20 a.m. Pulse 62 in fifteen seconds. Injected under skin of abdomen 1 cc. of solution of bufagin = 0.25 mg.

11.20 a.m. Pulse 62 in fifteen seconds. Injected under skin of abdomen 1 cc. of solution of bufagin = 0.25 mg.

11.25 Pulse 70 in fifteen seconds. Determined by auscultation.

11.30 Pulse 60 in fifteen seconds.

- 11.34 Pulse 60 in fifteen seconds. Injected 1 cc. of solution of bufagin = 0.25 mg.
11.37 Pulse 47 in fifteen seconds.
11.43 Pulse 46 in fifteen seconds.
11.49 Pulse 46 in fifteen seconds. Legs give way; lies on its side.
11.53 Pulse 44 in fifteen seconds. On auscultation heart sounds very loud and powerful.
11.56 Pulse 38 in fifteen seconds.
12 m. Pulse 38 in fifteen seconds.
12.03 p.m. Pulse 50 in fifteen seconds.
12.10 Pulse 52 in fifteen seconds.
12.15 Pulse 60 in fifteen seconds.
12.30 Pulse 62 in fifteen seconds. Recovered, eating hay.

Experiment 9. Male rabbit 1740 grams.

May 25. 12.25 p.m. Pulse 45 in fifteen seconds. Injected intraperitoneally 1.5 cc. of solution of bufagin = 1.12 mg.

12.40 Pulse 30 in fifteen seconds. Depressed; lies quietly in corner of cage.

5.00 Pulse 45 in fifteen seconds. Normal again.

May 26. 2.05 Pulse 30 in fifteen seconds. Hypodermic injection of solution of bufagin 4 cc. = 3.0 mg.

2.07 Pulse 60 in fifteen seconds. Passes urine.

2.30 Pulse 30 in fifteen seconds. Lies on its side. Respirations twenty in fifteen seconds.

2.50 Pulse 40 in fifteen seconds.

2.55 Pulse 30 in fifteen seconds. Respirations very shallow.

3.00 Pulse 28 in fifteen seconds.

3.13 Pulse 12 in fifteen seconds. Staggers. Slight convulsion noted. Respirations eight in fifteen seconds.

3.22 Pulse 18 in fifteen seconds. Frequent and violent clonic convulsions.

3.25 Respiration stops. Pupils widely dilated and eyes protruding; heart beat not felt, but on auscultation with stethoscope fibrillary contractions of heart seem to be heard, which soon cease. On autopsy, viscera very pale; ventricles firmly contracted in systole.

Experiment 10. May 29, 1911 Black cat. 2 kg.

12.44 p.m. Injected under skin of abdomen 5 cc. of solution of bufagin = 3.75 mg.

12.48 Vomits. Voids feces.

12.52 Clonic convulsions.

12.53 Pupils widely dilated. Death.

Experiment 11. November 23, 1911 Dog, 3.5 kg.

2.00 p.m. Tube passed into stomach and 4 mg. bufagin in 2½% alcohol solution introduced.

2.15 Animal lies quietly in corner of room.

2.30 Profuse vomiting and salivation. Depressed. One hour later complete recovery.

Experiment 12. June 6, 1911 Dog, 7.9 kg.

10.40 a.m. Pulse 36 in fifteen seconds. Injected 6 cc. solution of bufagin = 3.6 mg.

10.50 Pulse 36 in fifteen seconds.

11.00 Pulse 36 in fifteen seconds.

11.05 Pulse 36 in fifteen seconds. Injected 4 cc. solution of bufagin = 2.4 mg.

11.20 Pulse 24 in fifteen seconds.

11.25 Vomits.

11.35 Pulse 16 in fifteen seconds. Very irregular heart beat. Animal very sick.

11.55 Vomiting and trembling; then convulsions and death.

Experiment 13. Dog, 6 kg.

May 31, 1911. 1.35 p.m. Pulse 36 in fifteen seconds. Injected 10 cc. of solution of bufagin = 2.5 mg.

1.40 Pulse 30 in fifteen seconds.

1.43 Pulse 30 in fifteen seconds.

1.45 Pulse 32 in fifteen seconds. Injected 10 cc. of solution of bufagin = 2.5 mg.

1.48 Pulse 30 in fifteen seconds.

1.50 Pulse 35 in fifteen seconds.

1.55 Pulse 32 in fifteen seconds.

2.00 Pulse 34 in fifteen seconds.

2.05 Pulse 32 in fifteen seconds.

2.15 Pulse 32 in fifteen seconds.

2.30 Clonic convulsions.

June 1. 1.50 Recovered; apparently perfectly normal. Pulse forty in fifteen seconds.

Effect on man. Having studied the effects of bufagin in various doses on the lower animals, we proceeded to try the effect of small

injections of the drug on ourselves and on some of our colleagues. In this place we may merely mention that from injections of 1 mg. doses of bufagin in 5 per cent alcoholic solution (2.5 cc.) on ourselves and other normal subjects, we have noted a definite rise in systolic blood-pressure (from 10 to 25 mm.) and an increase in pulse-pressure (from 10 to 25 mm.) as measured with the Erlanger and Riva-Rocci instruments, and a slowing of the pulse-rate of from four to twelve beats a minute. There were no untoward effects noted, and there was no local irritation except the stinging sensation caused by the alcohol, which was no greater than in control injections of solutions containing the same quantity of alcohol only.

Drs. Macht and Rowntree of this Laboratory are at present engaged in a clinical study of the action of bufagin in pathological cardiac cases in the Johns Hopkins Hospital, and the results of their observations will be published in due time.

Effect of Bufagin on animals under anaesthesia. 1. *General action on the circulatory apparatus.* If we anesthetize a dog and inject a small non-toxic dose of bufagin (0.2 mg.) into one of its veins the blood-pressure tracing will show the following changes. Immediately or very soon after the injection there occurs a small rise in the blood-pressure with a normal or slightly accelerated pulse rate. This brief preliminary effect soon passes into what we have called the first or therapeutic stage in which we have a blood-pressure above the normal and a distinctly slower pulse rate with the amplitude of the pulse waves distinctly increased. If the dose injected is small the vagus effect gradually wears off and the curve assumes its normal character. If the dose be larger, the first stage passes into the second which may be termed the stage of excessive vagus stimulation. The pulse now becomes very slow, thirty or even twenty beats in the minute and the pulse waves are enormously increased in amplitude. The blood-pressure still remains high. The slowing of the heart beat may, however, be so extreme that the pressure falls below the level of the first stage in spite of the peripheral arterial constriction.

If during this stage the heart be exposed as was done in our

experiments with artificial respiration and curare, a true heart block may occasionally be observed, the auricles and ventricles beating in the ratio of 2 : 1, 3 : 1, or even 4 : 1. In exceptional cases, the heart may be permanently arrested in this stage, in which event, the ventricle will usually be found in diastole. More usually, however, and especially if more bufagin be injected, the action passes on to the third or toxic stage. The pulse now becomes very rapid and the blood-pressure rises to a higher level. Soon irregularities of the heart beat are noted and the blood pressure begins to fluctuate. The heart muscle is now seriously poisoned. Presently the pressure drops suddenly to near the zero level and the heart comes to a standstill. After such a fatal drop in the arterial pressure the left ventricle of the heart is almost invariably found to be firmly contracted in systole. The action of bufagin as above described coincides with that of the digitalis series whose action on the circulatory apparatus was first clearly classified in this manner by Cushny.

We have found that the same course of events follows the administration of bufagin hypodermically, intramuscularly and by mouth except that in the last case a slightly larger dosage is required to produce a given effect in a given time on account of the slowness of absorption.

The following protocols will illustrate the course of events above described. Fig. 6 contains parts of a kymograph tracing illustrative of the various stages in the action of bufagin on the circulation.

Experiment 14. March 20, 1911 Dog, 7.8 kg. Manometer connected to right carotid artery; injection cannula in left femoral vein; ether anaesthesia; curare; tracheal cannula; artificial respiration.

TIME	PULSE RATE	BLOOD PRESSURE IN MM.	REMARKS
	<i>per min.</i>		
10.00 a.m			Experiment begins.
10.01	84	120	
10.02	84	126	Injected bufagin 1 cc. = 0.000224 gram.
10.02½	84	126	Injection ends.
10.03	84	140	
10.10	90	116	Injected bufagin 2 cc. = 0.000448 gram.
10.10½	90	116	Injection ends.
10.11	90	124	
10.12	94	126	Respirations increased in rate and amplitude.
10.18	88	114	
10.18½	88	114	Injected bufagin 1 cc. = 0.000756 gram. (Stronger solution).
10.19	88	120	Injection ends.
10.19½	64	128	Vagus effect begins, slowing of rate, and greater amplitude.
10.20	64	126	
10.20½	32	126	Full vagus effect, pulse waves of great amplitude (20 mm.), extreme slowing,
10.21	32	128	
10.22	32	142	Respiration rapid.
10.23	32	144	
10.24	32	144	
10.30	32	112	
10.31	32	100	
10.33	32	104	
10.34	32	108	
10.35	110	140	Vagus effect ends, pulse waves of small amplitude, pulse very rapid.
10.36	118	118	
10.37	118	120	
10.42	122	140	Heart beat irregular.
10.42½	122	150	
10.43	122	130	
10.44	122	120	
10.45	122	120	
10.49	122	96	
10.50	122	96	Slow injection of bufagin 2 cc. = 0.001512 gram.
10.51	120	100	
10.51½	120	132	
10.52	120	150	
10.52½	120	164	Injection ends.
10.53	136	180	
10.55	136	190	
11.00			Sudden drop, stoppage of heart, death.

Experiment 15. Dog, 8.0 kg. Manometer connected to left carotid artery; injecting cannula in it femoral vein; ether; tracheal tube.

TIME	PULSE RATE	BLOOD PRESSURE IN MM.	REMARKS
	<i>per min.</i>		
11.00a.m			Experiment begins.
11.01	80	104	Respirations sixteen to one minute.
11.05	80	104	Injection of bufagin 0.5 cc. = 0.000112 gram.
11.05½	84	106	
11.06	84	124	
11.07	88	124	
11.10	88	126	
11.11	88	128	
11.12	88	130	
11.13	88	132	
11.19	88	136	
11.20	76	120	Vagus effect noticeable, slowing of rate. Respirations deeper.
11.21	66	112	
11.22	68	106	
11.23	68	96	
11.25	68	84	
11.29	68	90	
11.37	68	86	Injections of bufagin 1 cc. = 0.000224 gram.
11.38	76	100	Blood pressure rising.
11.38¾	44	110	Marked vagus effect, pulse waves of great amplitude.
11.40	44	112	
11.42	32	106	
11.54	54	110	
11.55½	54	130	Injections of bufagin 1. cc = 0.000224 gram.
12.00	30	130	
12.01p.m.	30	130	
12.10	30	122	
12.10¼			Injection of bufagin 10 cc. = 0.002240 gram begins.
12.11		132	
12.11½		144	
12.12		164	
12.12¾			Injection ends.
12.13		144	
12.15		110	Pulse waves too small and too rapid to be counted. Respirations powerful and abdominal in type.
12.16		140	
12.16½			Sudden drop of pressure. Death.

Experiment 16. Dog, 3.9 kg. Manometer connected with rt. carotid; injection cannula in left femoral vein; ether anaesthesia; tracheal tube.

TIME	PULSE RATE	BLOOD PRESSURE IN MM.	REMARKS
	<i>per min.</i>		
4.00p.m.	90	112	Respiration thirty in one minute.
4.02	90	112	
4.05	90	112	Injection of bufagin 2 cc. = 0.0015 gram, very slowly.
4.08½			Injection ends.
4.08	90	112	Respirations, thirty.
4.09¼	90	132	
4.08½	92	148	
4.10½	40	150	Very fleeting vagus effect, lasting about thirty seconds.
4.11			Pulse gets very rapid, small, and <i>irregular</i> .
4.12	124	126	
4.13	132	144	
4.14	126	134	
4.15¼	126	160	
4.16			Sudden drop of blood pressure. Death.

2. *Analysis of the action on the circulatory apparatus. The vagus effect.* The slowing of the pulse rate and the increase in the amplitude of the pulse wave noted in the latter part of the first stage and more especially throughout the second stage of the action of bufagin are consequences of the stimulating action of the drug on the cardio-inhibitory center as may be easily demonstrated by cutting the vagi, see Protocol of Experiment 17, or by administering atropine. In either case the inhibition is at once removed and the pulse curve assumes the well known characteristics of lack of vagus control. The transition from the first stage, or stage of moderate slowing, to the second stage of excessive vagus action is usually abrupt, and (see A, Fig. 7) the decrease in pulse rate is then *exactly one-half* of what it was just prior to the transition.

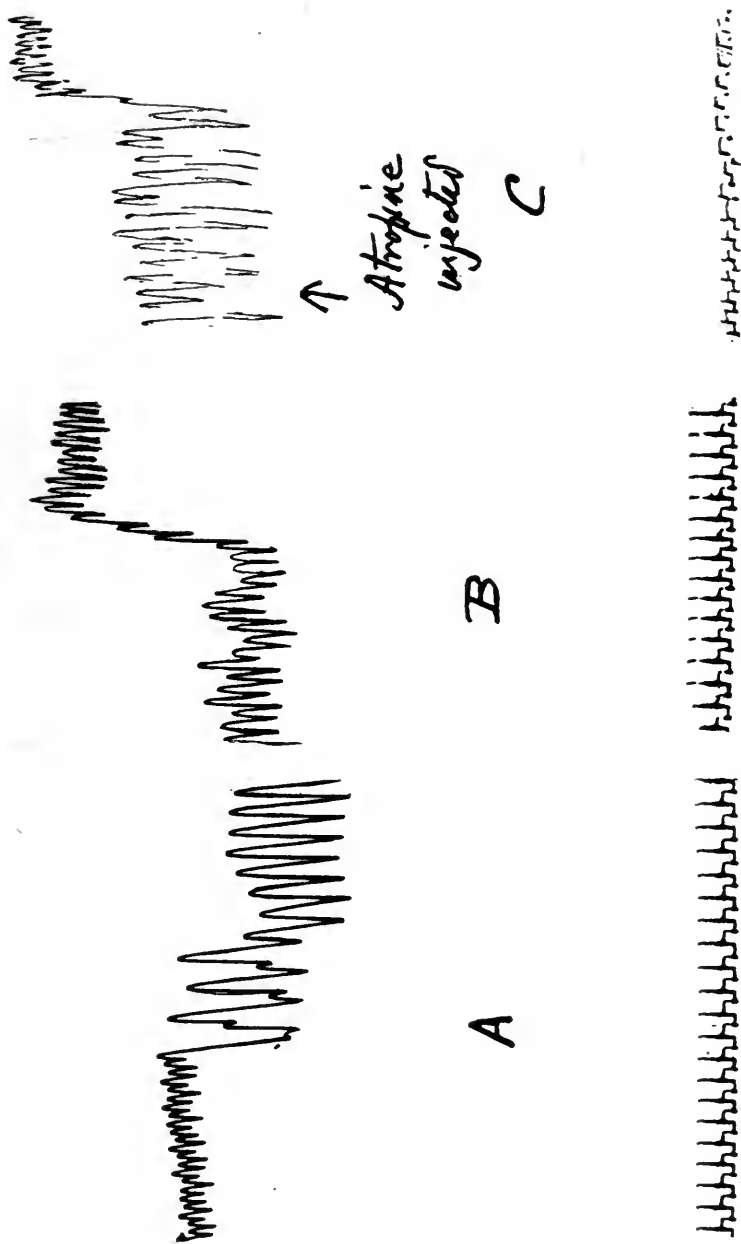


Fig. 7. Dog (7 kilos). Ether. Showing effect of 2.1 mg. of bufagin in divided doses intravenously. At A abrupt transition from first to second stage, at B five minutes later, abrupt transition to a condition similar to the first stage of the action of bufagin. C is part of a graphic record obtained from another dog showing the effect of 2 mgs. of atropine in abolishing the stage of extreme inhibition.

Experiment 17. March 23, 1911 Dog, 8.56 kg. Manometer connected to right carotid artery; injection cannula in left femoral vein; ether anaesthesia; tracheal tube; curare; artificial respiration.

TIME	PULSE RATE	BLOOD PRESSURE IN MM.	REMARKS
	<i>per min.</i>		
3.00p.m.			Experiment begins.
3.01	80	116	Respiration forty to the minute.
3.05	80	32	Curare injected in slightly excessive dose.
3.20	90	52	
3.21	90	62	Injection of bufagin 2 cc. = 0.001512 gram begins.
3.22	110	112	
3.23	108	144	
3.23½	104	176	Injection ends.
3.24	84	190	
3.26	84	190	Vagus effects noticeable.
3.30	52	206	Marked vagus effect, very long and slow sweeps of the writing pen.
3.34	52	156	
3.35	68	156	Right vagus cut.
3.36	130	206	Left vagus cut.
3.37	130	200	

The period of marked inhibition (2nd stage) sooner or later gives way to a stage of fast pulse and higher arterial pressure, the transitions to this stage being generally as abrupt as was the change from stage one to that of the slow pulse. (See *D*, Fig. 6 and *B* Fig. 7.) The events as here described are seen when considerable doses of bufagin are injected *intravenously*. The stages are crowded together, so to speak, the stage of marked inhibition may continue for only fifteen or twenty minutes, while in the un-aesthetized animal which has received the drug by intramuscular injection it may last for hours. The cause of the abrupt change from the second to the third stage may be assumed, as in the case of the members of the digitalis series, to be due not to a paralysis of the mechanism of the vagus, but to be indicative rather of an increased state of irritability of the musculature of the heart. The irritability of the heart reaches such a degree that the normal impulses of the vagus are inadequate to produce their usual

effect. This explanation was first offered by Cushny⁵³ for the analogous phenomenon seen in the digitalis series and was further elaborated later by Lhotak von Lhota.⁵⁴ Like other observers⁵⁵ who have studied digitalis-like bodies we have also found that, in the third stage of poisoning with bufagin, that is, at the time when the vagus mechanism is apparently paralysed, electrical stimulation of the vagus will, after a brief refractory period, cause marked inhibition of the heart with a fall of blood-pressure.

Action on the blood-vessels and other structures containing plain muscle. The marked rise of blood-pressure after injections of solutions of bufagin is due in some measure to the peripheral vaso-constrictor action of the drug. This action was well illustrated in the perfusion experiments with frogs and toads described in an earlier section of our paper. It is also illustrated in the action of the drug on the blood vessels of a loop of the dog's jejunum as measured with the intestinal plethysmograph (see Fig. 8). It may also be mentioned here that mere inspection of the contracted blood-vessels of the intestines in the stage of high blood pressure convinces us that vaso-constriction is a marked element in the rise of blood-pressure. The vaso-constriction was also noted in experiments made with the kidney enclosed in the oncometer, when it was seen that the *first* or immediate effect of an injection of bufagin is to cause a diminution in the volume of that organ.

In further illustration of the peripheral vaso-constrictor action of our drug we may cite the results of an experiment on a decerebrate cat with pithed cord (see Experiment 18). Through a misunderstanding the animal had received an excessive amount of morphine prior to the anaesthesia with ether and this together with a considerable loss of blood during the operation gave us rather a poor subject for the experiment. Under the conditions of the experiment, even though the arterial pressure did not rise to as high a level as might be expected (from 42 to 72 mm.) we may still believe that the observed rise was in a large measure due to an action on the peripheral vessels.

⁵³ Journ. of Exp. Medicine, ii, 233, 1897.

⁵⁴ Arch. f. Exp. Pathol. u. Pharmacol, lviii, 350, 1908.

⁵⁵ See Dale and Laidlaw. Action of Apocynum, Heart, i, 154 (1903-10).

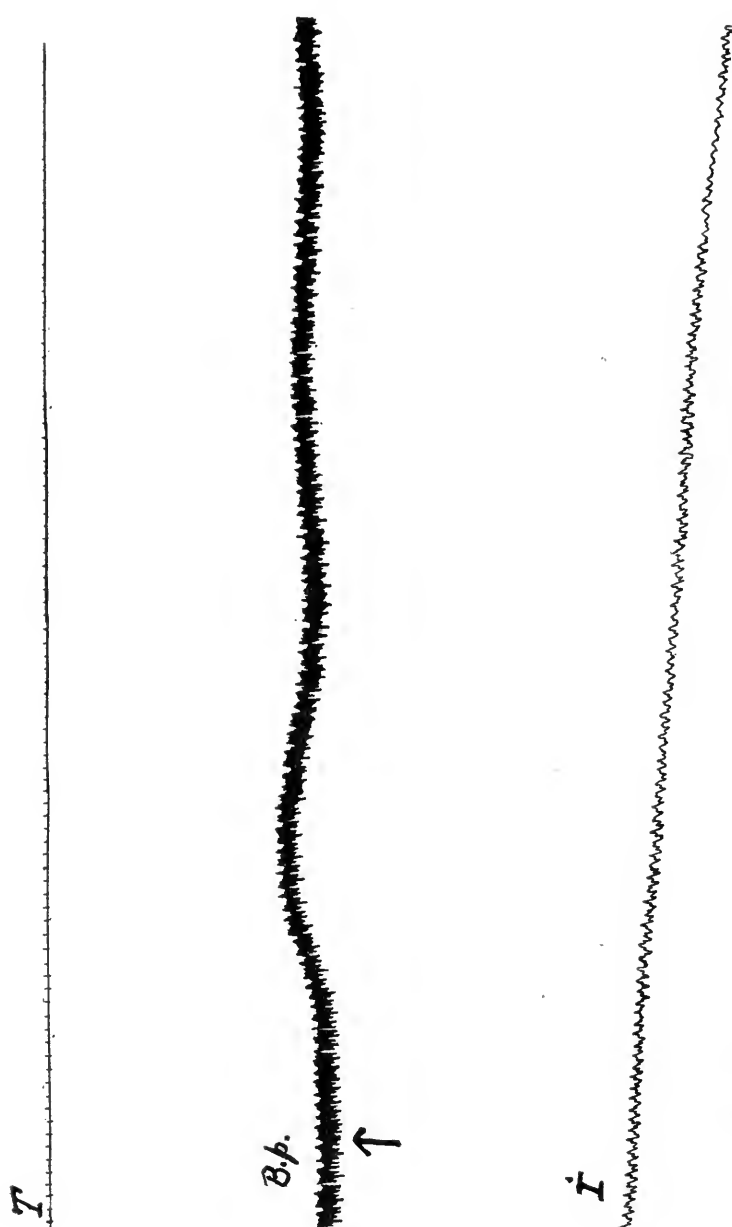


Fig. 8. Dog (7 kilos). Ether. T = time in seconds, $B. p$ = carotid blood-pressure, I = intestinal plethysmograph. At \uparrow 1 cc. = 0.34 mg. bufagin injected into right femoral vein.

Experiment 18. March 31, 1911 Cat, 2.8 kg. Ether and morphine; carotids and vertebral arteries tied off and animal decerebrated; spinal cord destroyed; manometer in right carotid artery; injections through left femoral vein; curare; artificial respiration.

TIME	PULSE RATE	BLOOD PRES- SURE IN MM.	REMARKS
	<i>per min.</i>		
11.00a.m	84	44	Experiment begins. Head removed and cord destroyed at 10.50.
11.03	84	44	
11.05	84	44	
11.07	84	40	
11.10	84	40	
11.13	84	42	
11.15	84	40	
11.15½			Injections of bufagin 1 cc. = 0.0007 gram begins.
11.16	90	46	Injection ends.
11.17½	108	54	
11.18	100	64	
11.18½	100	66	
11.22	92	50	Injection of bufagin 2 cc. = 0.0014 gram.
11.22½	92	44	
11.23	114	64	
11.23¾	114	72	
11.24	114	72	
11.24½			Sudden drop. Exitus.

The question as to whether the local vaso-constricting action of bufagin is exerted on the arterial musculature itself or on a nervous mechanism distal to the spinal cord was studied by Dale's⁵⁶ method. After the injection of a quantity of ergotoxine sufficient to abolish entirely the vaso-constrictor effect of epinephrin so that an injection of this substance gave the paradoxical fall of pressure, it was found that small doses of bufagin produced their usual effect in raising the arterial pressure. From this we concluded as was done by Dale and Laidlaw⁵⁷ under similar circumstances in their study of Apocynum, that bufagin acts

⁵⁶ Biochemical Journ., ii, 246, 1907.

⁵⁷ Heart, i, 138, 1909-10.

directly upon the plain muscle and not upon the nervous elements of the blood vessels.

The plain muscle of the rabbit's jejunum, of the pig's jejunum and of the isolated horn of the rabbit's uterus was in each case found to be stimulated to contraction by adequate quantities of bufagin. See Fig. 9.

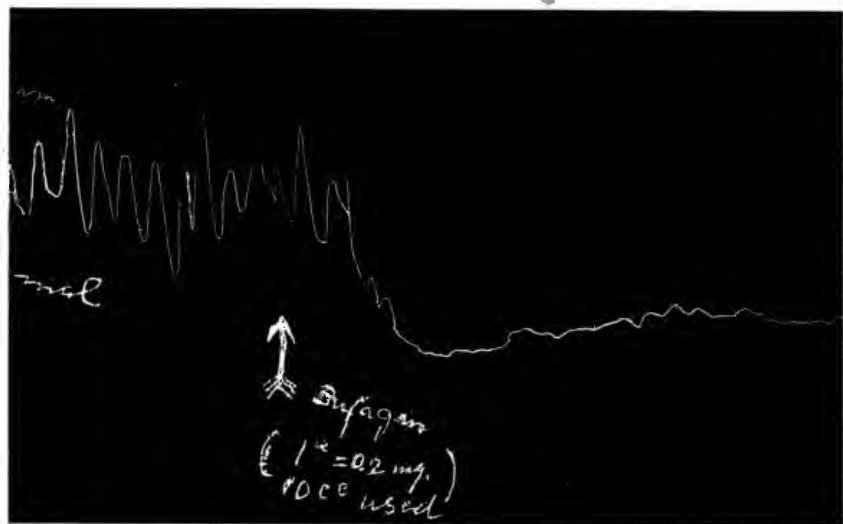


Fig. 9. Effect of bufagin on plain muscle. Isolated loop of rabbit's jejunum. Downstroke of lever indicates contraction. At \uparrow 2 mg. 10 cc. of solution were added to the warm oxygenated Locke's solution 200 cc. in which the loop was suspended.

The effect on the heart. Our experiments with the excised hearts of frogs, terrapins and toads have already indicated that bufagin has a powerful action directly on the heart itself. In those experiments we noted the great increase in tonicity and contractility, as well as the slowing of the heart beat, and, in the case of the toad, *Bufo Agua*, we found evidence of an effect on the bundle of His, causing a disturbance in conductivity and resulting in a heart-block.

The action of bufagin on the mammalian heart was studied by observation of the exposed heart in situ, by an analysis of blood-

pressure curves, and by means of tracings obtained with Cushny's modification of the Roy-Adami myo-cardiograph,⁵⁸ with the cardiac plethysmograph, and with Henderson's⁵⁹ cardiometer, an instrument well adapted to the study of ventricular volume change.

The effect on the heart after moderate doses of bufagin which result in the first or therapeutic stage, may be briefly summarized as follows:

There is a distinct increase in the tonicity of the heart-muscle (see Fig. 10), a slightly heightened irritability, and a decided increase in its contractility. The ventricular out-put is very much augmented, and this is due in part to greater tonicity and stronger contractions of the heart-muscle, and in part to a more complete relaxation in diastole (see Figs. 11 and 12⁶⁰).

The most striking effect on the heart-muscle in the second stage of the action of bufagin is the change in its conductivity, which finally leads to heart-block as already noted. This stage, as was pointed out previously is characterized by a marked action on the cardio-inhibitory centre. The contractile power of the heart is still unimpaired, but from the point of view of therapeutic use, this stage must be regarded as one fraught with danger.

In the toxic stage, the heart-muscle is poisoned, as may be seen from the disturbance of all its functions. The normal impulses from the vagus are inoperative, the rhythm of the heart-beat is impaired and its excitability is greatly increased, as may be seen from the extremely rapid and irregular pulse, with extra systoles. *Pulsus alternans* appears, as an indication of weakened contractile power. The contractions are small and irregular. The ventricular output is diminished and there is a relaxation in the tone of the muscle.

⁵⁸ Heart, ii, 1, 1910.

⁵⁹ Americ, Jour. Physiol., xvi, p. 352, 1906.

⁶⁰ We are indebted to Dr. G. S. Bond for kind assistance in performing this experiment.

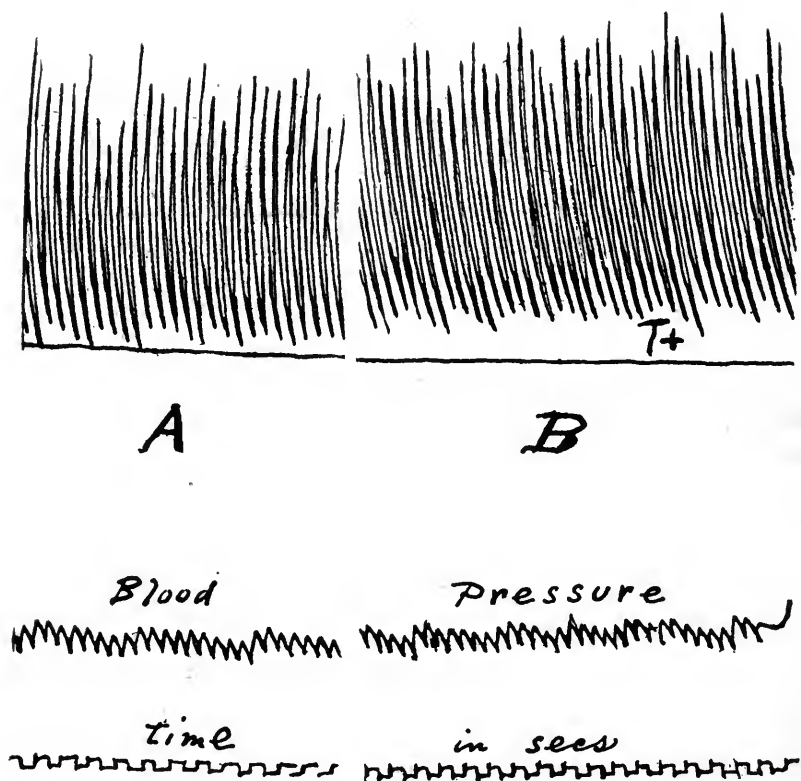


Fig. 10 Dog (9.15 kilos). Trichlor-tertiary-butyl alcohol. Ventricular tracing with Henderson's cardiometer, showing increase in tonicity in B. Upstroke = systole, A, normal; B, thirty seconds after injection of 0.2 mg. of bufagin. Early in the first stage of the action of the drug.

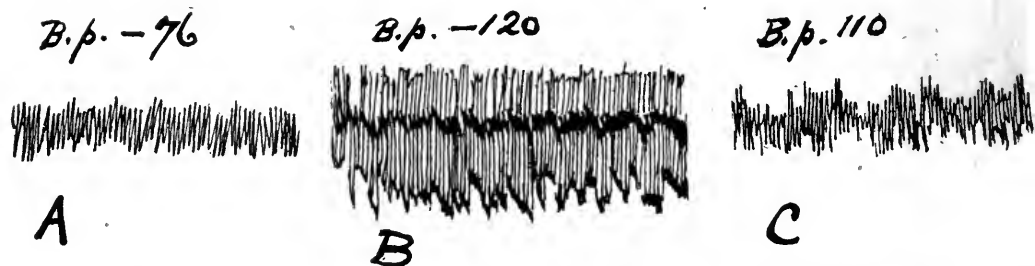
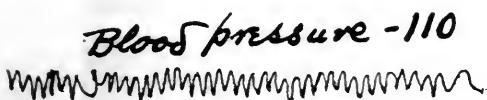
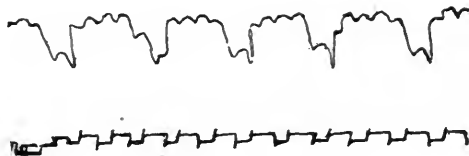
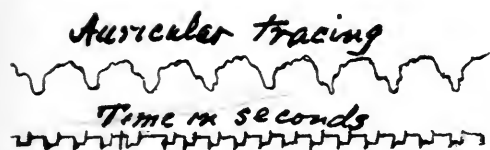


Fig. 11. Dog (9.5 kilos). Ether and Trichlor-tertiary-butyl alcohol. Myocardiographic Record. A, normal; B, two minutes after intravenous injection of 1.5 mg. bufagin; C, Toxic stage after further injections totaling 3.5 mgs.



A

B

Ventricular Tracing

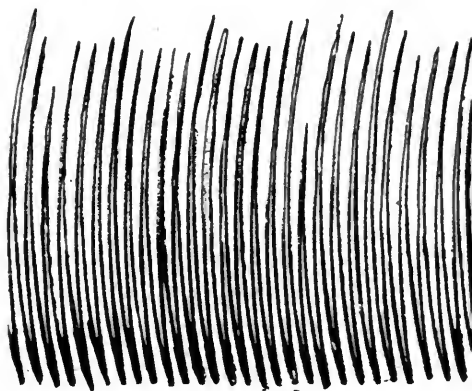
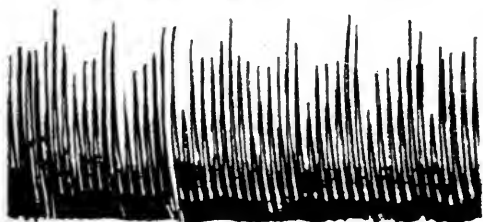


Fig. 12. Dog (6 kilos). Anaesthetized with Trichlor-Tertiary-Butylalcohol. Upper tracing gives the auricular contractions, the lower tracing is the cardiometer-record (Henderson) of the ventricular volume. Downstroke = systole. A, Normal. B, three minutes after injection into left femoral vein of solution containing 0.4 mg. of bufagin. Bloodpressure recorded from the left carotid artery.

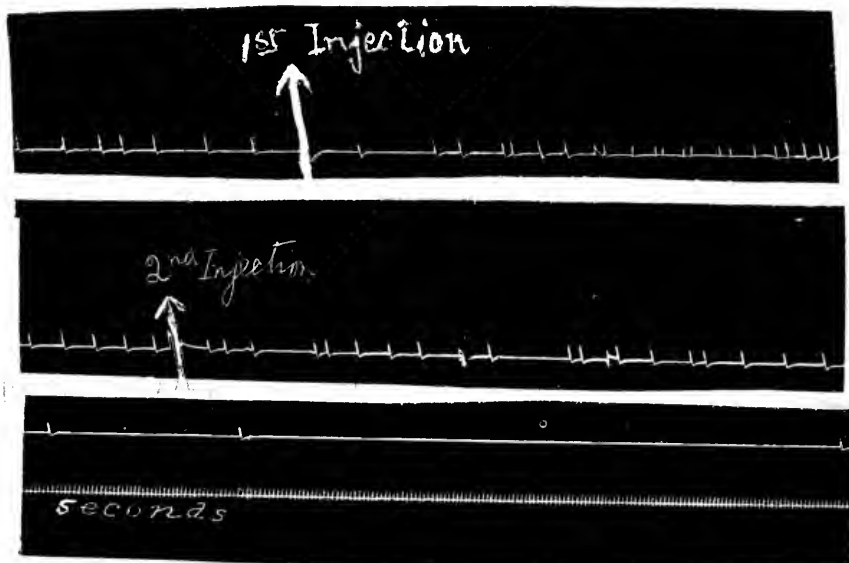


Fig. 13. Cat (1.6 kilos). Anesthesia with Trichlor-tertiary-butyl alcohol. Drop record of flow of urine. 1st injection into left jugular = 0.1 mg. Second injection five minutes later = 0.1 mg. Third line of drop record shows partial suppression of urine following repeated injections aggregating 0.6 mg. Last line record in seconds.

Diuretic action.

We have studied the diuretic action of the drug on the rabbit and the cat and have found that it causes an increase in the flow of urine in both animals. A rabbit weighing 1.85 kilos, anesthetized with paraldehyde and urethane had normally a urinary flow of seventeen drops per minute as measured by a drop record from a bladder cannula. After the injection of 0.5 mg. of bufagin into the left jugular vein the rate of flow quickly increased to twenty-nine drops per minute which rate was maintained for nearly five minutes. The carotid blood pressure during this time increased from 80 mm. to 100 mm.

In the cat small doses of the drug quickly induce a free flow of urine, as may be seen from a study of the protocol of Experiment 17 and of Fig 13, with this animal as with the rabbit, toxic doses

cause a diminution of the flow which finally passes into complete suppression. These positive results in the way of diuresis may be gained with therapeutic doses.

Oncometer and blood pressure experiments on the cat during diuresis after bufagin have yielded the following results: After small doses there is seen at first, though not invariably, a diminution in the volume of the kidney. This is soon followed by a dilatation of the renal blood vessels, as is shown by an increase in the volume of the kidney, which goes hand in hand with a rise of blood-pressure and a marked increase in the flow of urine. After the administration of toxic doses the volume of the kidney decreases coincidently with the period of very high blood pressure and marked vaso-constriction, the flow of urine is lessened and soon complete suppression occurs (see Fig. 14).

It is evident from the above that small doses of bufagin cause a vaso-dilatation in the kidney at a time of constant or higher blood pressure and vaso-constriction elsewhere in the body. In this respect the drug acts like other members of the digitalis series whose behavior in this regard has been studied by Jonescu and Loewi.⁶¹

Experiment 17. Diuresis. Cat (1.6 kilos). Anaesthetized with trichlor-tertiary-butylalcohol. Injection cannula in left jugular vein. Cannula with funnel shaped end in bladder, so that lumen of bladder is reduced to the size of the cannula. Urethra ligated. Normal rate of dropping of urine = six per minute. Thirty seconds after injection of 0.1 mg. bufagin number of drops = twelve per minute. Three minutes later still twelve per minute. Five minutes later second injection of 0.1 mg. One minute later flow = nine drops per minute, three minutes later ten drops per minute, five minutes later six per minute. Then repeated injections at intervals of about one minute until the whole quantity from the beginning totaled 0.8 mg. The result of this toxic dose was gradual suppression of the urinary flow terminating in complete anuria.

⁶¹ Arch. f. Exp., Pathol. u. Pharmakol, lix, 71, 1908.

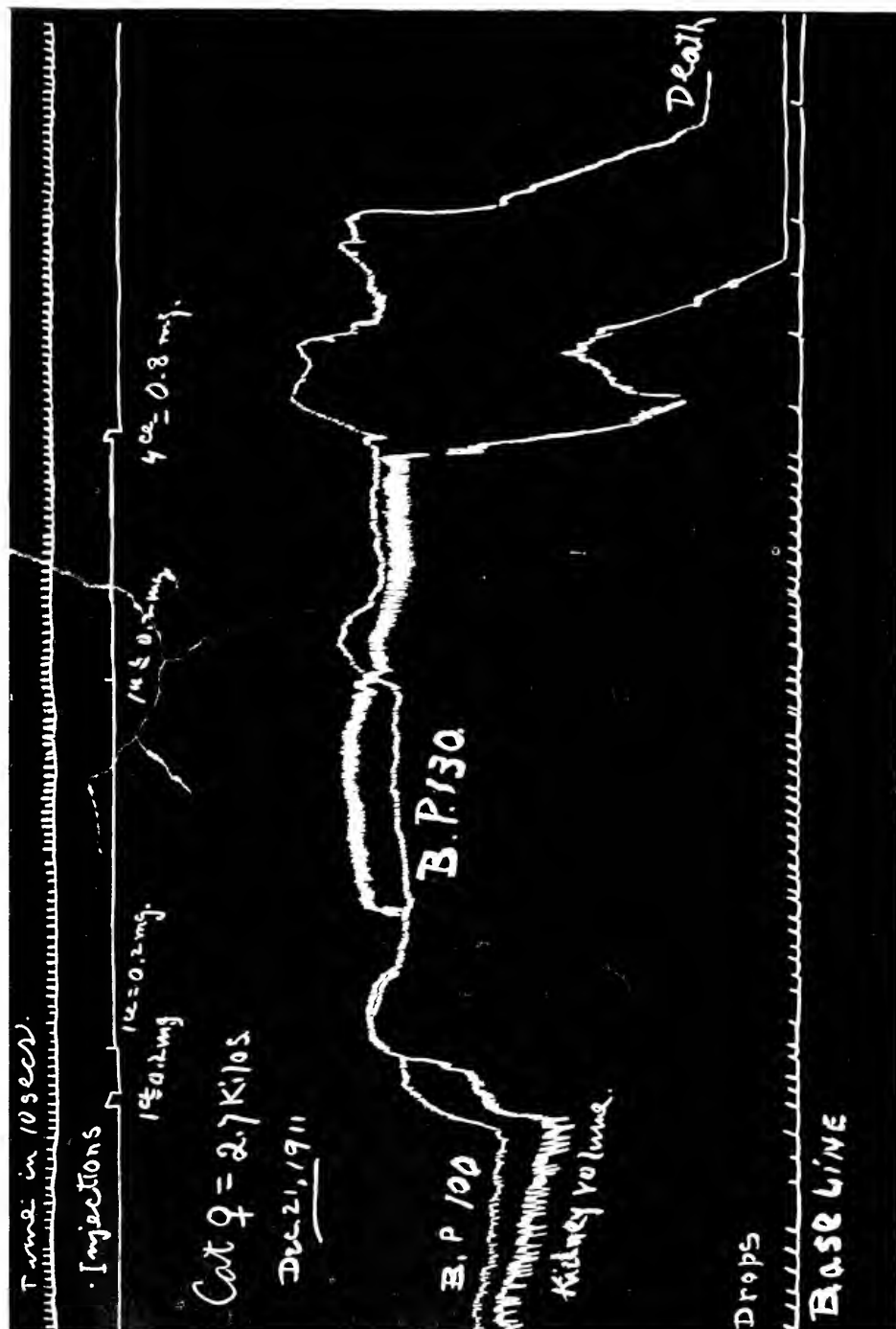


Fig. 14. Cat (2.7 kilos). Trichlor-tertiary-butylalcohol. Carotid blood pressure, injection cannula in left femoral vein. Urinary cannula in bladder. Left kidney in oncometer. Piston recorder. Drop record for urinary flow is base line of blood pressure. Time intervals = ten seconds. Dose of bufagin and times of injection indicated on second line.

VI. SUMMARY

1. From the secretion of the parotoid glands of the tropical toad, *Bufo aqua*, we have isolated two distinct, physiologically active crystalline principles.

2. It has been shown by chemical reactions and by analyses, by polarimetric observations, and by qualitative and quantitative physiological experiments that one of these substances is identical with dihydroxy-methyl-amino-ethylol-benzene (epinephrin, adrenalin, suprarenin).

3. It is calculated that the crude venom contains nearly seven per cent (6.72 per cent) of this amino alcohol.

4. By means of chemical reactions and analyses it has been shown that the venom also contains a crystalline principle which we have named bufagin.

5. Bufagin is dextrorotatory ($+11^\circ$), "neutral" in character, slightly soluble in water and readily soluble in a number of organic solvents. Its melting point is $217-218^\circ\text{C}$. Its elementary composition and molecular weight are represented by the formula $\text{C}_{18}\text{H}_{24}\text{O}_4$. Its behavior toward bromine shows that it does not contain an unsaturated carbon linkage of cholesterin.

6. The marked action of bufagin on the heart, cardio-inhibitory centre and musculature of the blood-vessels compel us to class this drug with the most efficient members of the digitalis series. Its action on the heart muscle is especially noteworthy. It increases its tonicity, the strength of its contractions and the ventricular volume output. Small doses of the drug cause a marked diuresis.

7. Bufagin does not appear to have a cumulative action and it may be administered hypodermically.

8. The toad, *Bufo aqua* is not at all immune to dihydroxy-methyl-amino-ethylol-benzene, but is relatively immune to bufagin.

9. The pharmacological properties of bufagin, its keeping qualities, its chemical purity and the consequent ease with which it lends itself to exact dosage are facts that urge a trial of this substance in therapeutics. The successful use of toad skins in the medicine of an earlier day also lends support to our suggestion.

A NOTE ON THE EFFECT OF NICOTINE INJECTION ON ADRENAL SECRETION

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The observations of Dreyer,¹ Tscheboksaroff,² and Asher³ have proved that adrenal secretion is under the control of the thoracico-lumbar autonomic (sympathetic) system. Langley and Dickenson concluded from their studies of the action of nicotine that it at first stimulates and later paralyzes sympathetic ganglia.⁴ Small doses of nicotine might, therefore, be expected to cause an increase of adrenal secretion by stimulating the ganglia occurring in the sympathetic supply of the adrenal glands.

The present investigation was undertaken to learn whether nicotine can in fact increase the discharge of epinephrin into the blood. Cats, under complete ether anaesthesia, were used for the experiments. The method of testing for the presence of epinephrin was that devised by Cannon and de la Paz.⁵ The longitudinal strips of intestinal smooth muscle were taken through a small abdominal incision from the animal later to be injected with nicotine. The strips were cut into convenient lengths and placed in aerated Ringer's solution kept at approximately 37° C.

The blood to be tested was removed, as in the experiments of Cannon and de la Paz, by introducing a fine catheter vaselined inside and out, through the left femoral vein into the inferior vena cava to a point immediately anterior to the opening of the adrenal

¹ Dreyer: Am. Jour. Physiol., 1898-99, ii, p. 219.

² Tscheboksaroff: Arch. f. d. ges. Physiol., 1910, cxxxvii, p. 103.

³ Asher: Zentralbl. f. Physiol., 1910, xxiv, p. 927.

⁴ Langley and Dickenson: Jour. Physiol., 1890, xi, p. 303.

⁵ Cannon and de la Paz: Am. Jour. Physiol., 1911, xxviii, p. 65.

veins. To the catheter was attached a small glass aspirator by which the blood was withdrawn.

Before injection of the nicotine solution a sample of blood (to be called "blood A") was extracted. The time of extraction was noted. The blood was transferred immediately to a beaker, defibrinated by whipping, and then set in a water-bath at 37° C. A cannula was now inserted into the right femoral vein and the solution of nicotine (Merck's) was allowed to run in slowly. The time of this injection was taken. After an interval varying usually between three and five minutes blood was again extracted by means of the catheter, from the same region in the inferior vena cava; the blood was defibrinated as before, and preserved at body temperature. This was "blood B." In some of the experiments a third extraction ("blood C") was made ten to twelve minutes after the end of the nicotine injection.

The several specimens of blood—A, B and C—were then tested for their content of epinephrin. The strip of intestinal muscle, attached to a writing lever, was suspended between two *serres fines* in a small cylindrical chamber through which air was passed. The chamber was surrounded by water at 37° C. The characteristic inhibition of the rhythmic contractions of the muscle, even when it has been for some time removed from the body, occurs at a dilution of adrenalin 1:2,000,000 in defibrinated blood.⁶

In testing the samples of blood the first step consisted in securing a record of the rhythmic contractions of the intestinal strip when surrounded by warm aërated Ringer's solution. The solution was then withdrawn by means of a fine pipette, and blood A was substituted. After the muscle had come to a condition of constancy, blood A was withdrawn, and blood B (removed from the animal after the nicotine injection) was substituted. The muscle was again allowed to reach a constant condition, whereupon blood B was replaced by blood A or by Ringer's solution.

The results which were secured are illustrated in the accompanying tracings.

The animal whose blood-reaction is presented in Fig. 1 received 0.0037 gram (0.75 cc. of 1 : 200) of nicotine. The registering

⁶ Cannon and de la Paz: Loc. cit., p. 69.

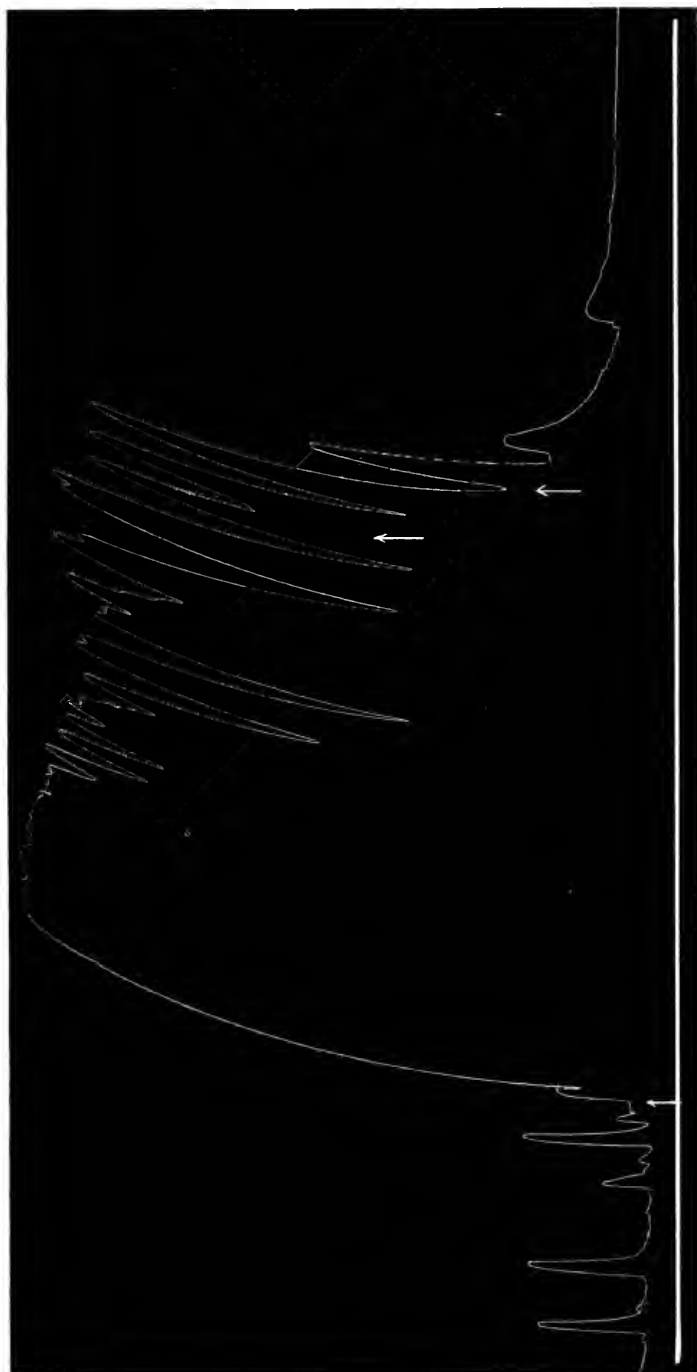


FIG. 1. Intestinal strip beating first in Kinger's solution. At x normal blood substituted; at y removed. At z blood from animal previously injected with 0.0037 gram nicotine was applied.

muscle was beating first in Ringer's solution. At *x* that solution was exchanged for blood A. The characteristic increase of tonus of the muscle, when freshly surrounded by blood, was at once recorded. At *y* blood A was removed, and at *z* blood B was substituted. The contractions were promptly inhibited, in a manner typical of the action of epinephrin. This observation was corroborated by other similar experiments, as shown in Table 1.

TABLE I

CAT	NICOTINE	SOLUTION	TIME OF EX-TRACTING BLOOD B AFTER INJECTING NICOTINE	ACTION OF MUSCLE STRIP ON ADDITION OF BLOOD B
	<i>grams</i>	<i>cc.</i>	<i>minutes</i>	
1	0.025	5 (1 : 200)	5 10	Slight slowing of rhythm Contractions ceased (inhibition)
2	0.015	3 (1 : 200)	4	Inhibition
3	0.015	3 (1 : 200)	4 10	Slowing Further slowing and loss of tonus
4	0.0075	3 (1 : 400)	5	Slight loss of tonus and inhibition
5	0.004	1.6 (1 : 400)	3	Loss of tonus. Inhibition. Occasional contraction
6*	0.0037	0.75 (1 : 200)	3	Loss of tonus. Inhibition
7	0.0037	1.5 (1 : 400)	1 8	Slight slowing Loss of tonus. Occasional contractions
8	0.0037	1.5 (1 : 400)	5	Loss of tonus. Inhibition. Occasional contractions
9	0.0027	1.1 (1 : 400)	3	Increased tonus. Contractions regular Dose subminimal?

* Fig. 1.

Magnus has shown⁷ that the action of nicotine (5 to 10 cg. added to 200 cc. of Ringer's solution) is to produce a brief inhibition of the rhythmic contractions of the isolated strip of intestinal muscle. Before the conclusion can definitely be drawn that epinephrin produces the inhibition obtained after injecting nico-

⁷ Magnus: Arch. f. d. ges. Physiol., 1905, cviii, p. 17.



FIG. 2. Intestinal strip beating in about 3.5 cc. normal defibrinated blood. At *x* and *y* and *z* nicotine added—in all more than 1 cc. of 1:200 solution.

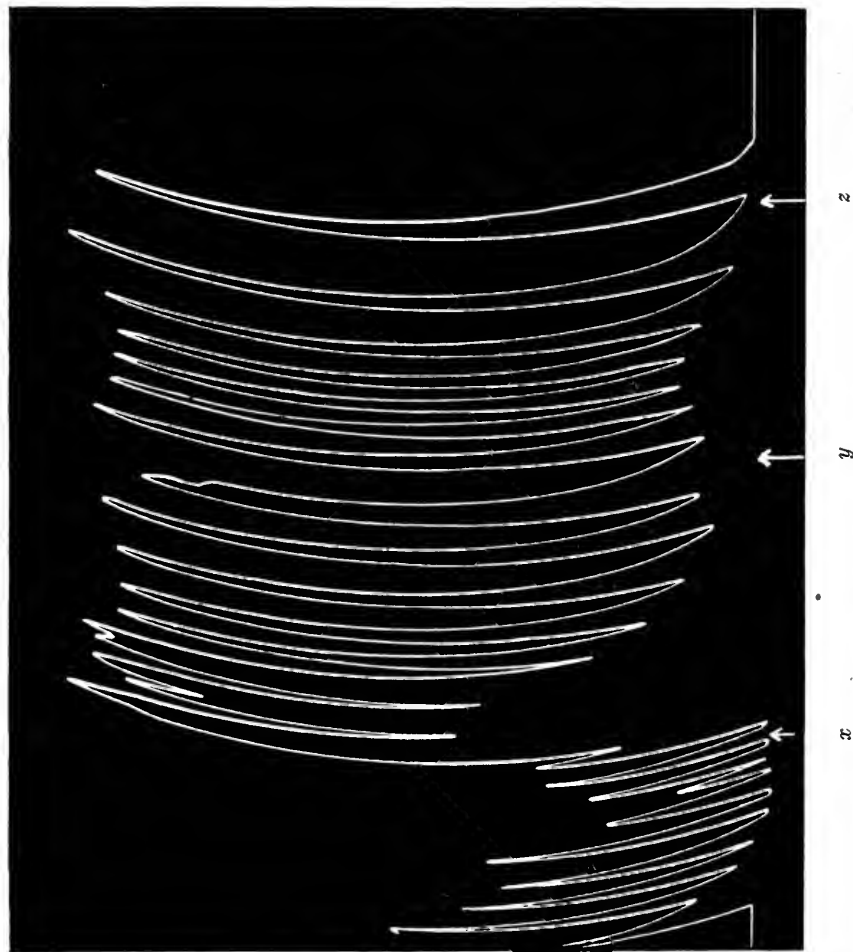


FIG. 3. Intestinal strip beating first in Ringer's solution. At x normal blood added, at y blood from decapsulated animal previously injected with 0.0075 gram nicotine was substituted. At z one drop of adrenalin (1:1000) was added.

tine, it is necessary to eliminate the possibility that the injected nicotine itself is the cause.

The effect of nicotine was tested by two methods. In the first, nicotine was added to the normal defibrinated blood in which the strip was contracting. In Fig. 2 is recorded the effects of small doses of nicotine on the contractions. At *x* and *y*, and *z*, nicotine was added, in all more than 1 cc. of a 1:200 solution. The amount of blood in the chamber was approximately 3.5 cc. When to this an amount of nicotine was added sufficient, if injected into a cat, to produce total inhibition of the contractions, there resulted not relaxation but increased tone and more rapid rhythm.

The second method of eliminating the possibility that injected nicotine caused inhibition of the pulsating strip was by testing the blood from decapsulated animals. Blood A was extracted as in previous experiments. The animals were then deprived of their adrenal capsules, and after twenty to thirty minutes they were given 0.0075 gram nicotine, a dose which, as Table 1 shows, is capable of producing complete relaxation of the test muscle. At the usual period after the nicotine injection blood B was extracted. In no case was there any loss of tone, slowing of the rhythm, or inhibition. In Fig. 3 these results are presented graphically. At *x* normal blood was added, at *y* blood taken from the animal after nicotine injection was substituted. The addition of one drop of adrenalin (1:1000) at *z* caused gradual loss of tone and inhibition, which proved that the muscle was still sensitive to the presence of epinephrin.

Nicotine, as used in these experiments, does not produce inhibition of the pulsating intestinal strip. Blood taken from near the openings of the adrenal veins after nicotine injection differs from blood removed from the same region before the injection, in producing inhibition of the pulsating strip. This difference does not appear if nicotine is injected after the adrenal glands have been removed. The conclusion is therefore justified that injection of nicotine in small amounts (0.0035 to 0.0075 gram in cats) results in augmented adrenal secretion.



QUANTITATIVE STUDIES ON THE GASTRO-INTESTINAL ABSORPTION OF DRUGS: II. THE ABSORPTION OF SODIUM IODIDE

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INTRODUCTION

The first of this series of papers¹ dealt with the quantitative absorption of phenol from the alimentary canal. In this, the second of the projected series of investigations on absorption, Dr. Sollmann proposed to me to apply the same plan of study to a substance which differs physically, chemically, pharmacologically and physiologically as widely as possible from phenol; and for this he chose sodium iodide. It was found that the course of absorption presented many points of similarity, especially the curious inhibition, following the rapid initial absorption. The mechanism of this inhibition, however, is quite different, the haloid salts producing a specific action in the permeability of the epithelium to haloid salts.

METHODS

In these experiments dogs and cats were used interchangeably, since the absorption in these animals did not differ materially. This will be discussed presently. All animals were in a stage of complete anesthesia before the experiment was begun. For dogs a preliminary hypodermic injection of morphin sulphate 20 mgms. per kilo was used, supplemented by ether. For cats, the usual laboratory anesthetic consisting of ethyl carbamate 20 grams, atropin sulphate 0.02 gram, and morphin sulphate 1.0 gram in 100 cc. of

¹ Sollmann, Hanzlik and Pilcher: J. Pharm. and Exp. Therap, 1910, i, p. 408.

water was used, in the dose of 3 cc. per kilo by rectum; and this too was supplemented by ether as necessary. In all cases tracheotomy was performed, the carotid artery (usually the left) being connected with a blood-pressure recording apparatus. Laparotomy was then done to expose the intestine. During the experiments all animals were kept warm, and the intact intestines were replaced in the abdomen and edges of the wound were closed. In all cases where experiments were performed with several loops, the intestine was doubly ligated into loops of equal length (15 cms.). Details other than these will be given in their proper place.

The injection of sodium iodide was accomplished by the use of a graduated burette. With a hypodermic needle attached to a rubber tubing leading from the burette, a definite quantity of a known sodium iodide (10 cc. per kilo of approximately 1 per cent) solution was injected directly into a loop or any portion of the gastro-intestinal tract, close to a ligated end. The needle puncture was ligated immediately upon withdrawal of the needle to prevent leakage of the iodide. The solution was allowed to remain the required length of time; the organ was then excised and the sodium iodide in the tissue and contents recovered according to a method previously described.² The amount recovered subtracted from the amount injected, indicated the amount of sodium iodide absorbed. All of the results are expressed in percentage, except in special cases where the difference will be noted.

Throughout the paper, I shall compare the iodide results with those described by us for phenol. The Roman numerals in the conclusions refer to the corresponding sections of our Phenol paper.

EXPERIMENTAL RESULTS

1. The identical behavior of cats and dogs

Comparing the average percentage of sodium iodide absorbed from the untreated gastro-intestinal tracts of cats and dogs, the conditions of the experiments otherwise being identical, it was found that:

² Hanzlik: J. Biol. Chem., 1910, vii, p. 459.

Ten cats absorbed from 31 to 84 per cent, an average of 58 per cent and eleven dogs absorbed from 32 to 86 per cent, an average of 61.34 per cent. The quantitative absorption of sodium iodide from the gastrointestinal tract in dogs and cats is practically identical. The small difference falls within the limits of error of experimentation and is smaller than the difference observed in animals of the same species.

The same conclusion applies to phenol (III),³ although the average absorption of phenol was not much more than half as great as that of the iodide.

2. Absorption of sodium iodide according to the site of the gastrointestinal tract

In order to determine this, various portions of the gastrointestinal tract were doubly ligated and injected with equal quantities of 1 per cent sodium iodide. At the end of a half hour sojourn each portion was excised and the absorbed sodium iodide determined. The results obtained are indicated in Table I.

TABLE I

Absorption of sodium iodide according to the site of the gastro-intestinal tract

SITE	PERCENTAGE OF SODIUM IODIDE ABSORBED				AVERAGE PER CENT OF NaI ABSORBED	
	Experiment I Dog	Experiment VIII Cat	Experiment XIII Cat	Experiment XV Dog		
Stomach.....	52.40	76.56	25.71	32.48	46.79	} 64.21 Average
Duodenum.....	41.27	90.90	57.10	74.35	65.91	
Jejunum.....	4.76	92.06	66.20	81.19	61.06	
Ileum.....	23.80	88.88	84.28		65.65	
Colon.....	34.43	58.73	34.28	58.11	42.15	
Average	32.02	81.43	53.51	61.53	56.31	

The average of all experiments for the three portions of the intestinal tract which comprise the small intestine is equal to 64.21 per cent; for the stomach 46.79 per cent and for the colon 42.15 per cent. Evidently then, the greatest average percentage of absorption of sodium iodide takes place along the course of the

³ The Roman numerals indicate the corresponding sections of our paper on Phenol-absorption.

small intestine between the pylorus and the colon. It is also very well indicated in our table that the duodenum, jejunum and ileum are in close harmony as to the average percentage of absorption. The same is true of the stomach and colon.

These conclusions can be confirmed by arranging the data in another way: If we assign to each portion of the gastro-intestinal tract in Table I, an arbitrary numerical position in the order of greatest average percentage absorbed, beginning with one, then a constant position is occupied by the various portions of the small intestine, viz., duodenum, jejunum, and ileum (i.e., 2.2, 2.5, 2.3 respectively) and the stomach and colon (i.e., 3.5, 3.7, respectively).

Conclusion: The experiments show that the "average absorption" of sodium iodide, in cats and dogs, is approximately the same for all divisions of the small intestine. The absorption from the stomach and colon is about a third lower than that from the small intestine. Individual experiments, however, do show unexplained variations from the average positions.

With phenol (III) the absorption was quantitatively identical for the stomach and intestines in dogs and cats.

3. *The extent of surface does not influence the percentage of absorption*

This was determined by placing equal quantities of sodium iodide into two ligated loops of different length in the same animal. The longer loop had about six times as much absorbing surface as the shorter loop.

At the end of a half hour the loops were excised and the sodium iodide estimated. The results are indicated in Table II.

TABLE II
Absorption of sodium iodide according to extent of absorbing area

LENGTH OF INTESTINE	PERCENTAGE OF SODIUM IODIDE ABSORBED			AVERAGE PER CENT NAI ABSORBED
	Experiment V	Experiment VI	Experiment XVII	
<i>cm.</i>				
90	52.70	62.70	85.47	66.95
15	44.40	60.00	87.18	63.86

In the three experiments performed upon cats and dogs differences were found to exist as to the quantity of sodium iodide absorbed in different animals, but the long and short loops of each individual showed a striking agreement.

Conclusion: The extent of the absorbing surface of the intestine has practically no effect on the quantitative absorption of sodium iodide.

Phenol (IX) gave the same results.

4. *The effect of different concentrations of sodium iodide on the absorption*

Solutions of sodium iodide of different strengths but containing the same absolute dose were prepared and injected simultaneously into loops of equal length into the same animal. These were left intact for half an hour, then excised and the sodium iodide determined. Table III indicates the results obtained.

TABLE III

Absorption of sodium iodide according to different concentrations

CONCENTRATION OF NaI USED	PERCENTAGE OF SODIUM IODIDE ABSORBED FROM SINGLE LOOPS			AVERAGE PER CENT NaI ABSORBED
	Experiment IX	Experiment X	Experiment XI	
<i>per cent</i>				
1	78.33	60.00	66.00	66.11
2	83.33	88.09	62.85	78.09
5	80.00	70.00	55.55	68.52
10	92.57	90.31	67.03	83.30
Average.....	83.56	77.10	62.86	74.01

The 2 per cent solution seemed to be better absorbed than either 1 per cent or 5 per cent in two experiments, but only one of these shows a marked difference. The result is, therefore, probably accidental. The 10 per cent solution always showed a greater absorption and the difference was fairly marked, averaging 83.3 per cent against 70.9 per cent with 1 per cent to 5 per cent solutions.

Conclusion: There is no material difference in the absorption of 1, 2 and 5 per cent solutions of sodium iodide. Ten per cent solution is absorbed somewhat more readily.

With phenol (VIIa), the undiluted substance was absorbed a trifle better than a 5 per cent solution.

5. Absorption of sodium iodide according to time of sojourn

This was deduced by the introduction of equal quantities of sodium iodide into several intestinal loops of equal length and the

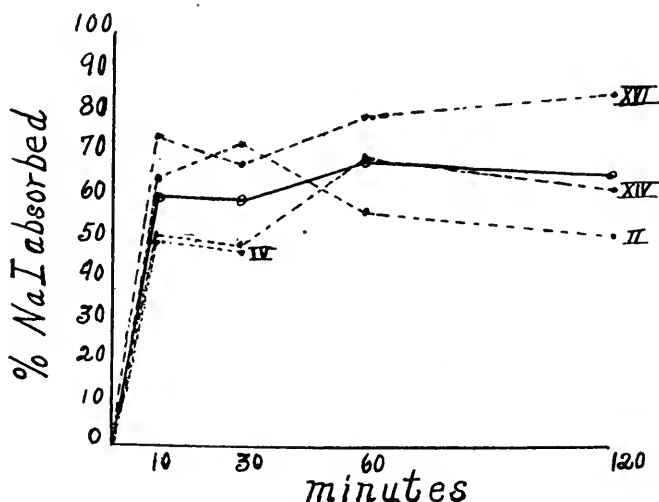


FIG. I. ABSORPTION OF SODIUM IODIDE ACCORDING TO TIME OF SOJOURN
The solid line represents the mean curve of the plotted experiments.

excision of these at successive intervals. The average percentage of absorption of the four experiments was 60.28 per cent at the end of ten minutes. This was unchanged (60.17 per cent) at the end of thirty minutes, increased to 69.57 per cent at the end of one hour and diminished to 68.03 per cent at the end of two hours. The result from the individual experiments as well as their averages are represented in Fig. I which was constructed from the data appearing in Table IV.

Conclusion: The absorption of sodium iodide from the intestine of cats and dogs is practically arrested at the end of the first

TABLE IV

Absorption of sodium iodide according to time of sojourn

TIME OF SOJOURN	AVERAGE PERCENTAGE OF SODIUM IODIDE ABSORBED FROM DUPLICATE LOOPS				AVERAGE PER CENT NaI ABSORBED
	Experiment II	Experiment IV	Experiment XIV	Experiment XVI	
Ten minutes.	64.25	50.00	51.47	75.39	60.28
Thirty minutes.....	73.85	48.61	49.00	69.23	60.17
One hour.....	58.70		70.00	80.02	69.57
Two hours...	52.40		63.26	88.44	68.03

ten minutes after injection. Up to the end of the first hour there is a very small further absorption. Between the first and second hour there is no further absorption. In other words, the absorption proceeds very rapidly during the first ten minutes, and is then practically arrested, leaving from 25 to 50 per cent of the iodide unabsorbed.

Bröking⁴ and Abderhalden and Kautzsch⁵ investigated the absorption of potassium iodide in the intact animal by the excretion in feces and urine, and found it to be rapid and nearly complete and which agrees with my observations. Friedman⁶ observed that when an aqueous solution of potassium iodide was applied per rectum in an animal the iodide was absorbed more rapidly than when given *per os*, as indicated by the excretion in urine. Inasmuch as the iodide was administered somewhat differently in his experiments no comparison can be made with my results.

Semmola⁷ quotes an interesting experiment which may have some bearing on these phenomena. If ferrocyanide is introduced into the intestine, it passes quickly into the urine; but the excretion stops after a time when ferrocyanide is still present in the intestine. In other words there is an inhibition of absorption.

The absorption of phenol (I) took a similar course.

The general phenomena of the course of absorption of sodium iodide as described in the preceding sections bear a close resem-

⁴ Bröking: Z. f. exp. Path. u. Therap, viii, p. 123.

⁵ Abderhalden and Kautzsch: Ibid., 1907, iv, p. 716.

⁶ Friedman: Diss. Giessen, 1910., cit. Z. Biochem. u. Biophysik, 1910, x, p. 619.

⁷ Semmola: Experimentelle Pharmakologie, 1890, p. 72.

blance to those observed with phenol. Especially striking is the resemblance between the curves of absorption as indicated by the "time of sojourn." This raises the question whether the mechanism is identical. That this is not so is shown in the following section.

6. *The effect of succeeding doses on the absorption*

The fact that phenol checked absorption in intestinal loops whose surfaces were prevented from coming in contact with preceding doses of the substance showed that it exerted its checking influence in some other way besides by local contact. Sodium iodide was investigated in the same direction with the following results:

Loops of intestine of equal length were ligated off as a preliminary step. Then each loop was injected with a 1 per cent solution of sodium iodide, left intact for ten minutes and excised before the next loop was injected. In Experiment XLVI duplicate loops were simultaneously injected but in all other respects the experiment was carried out as in III, VII and XII where only single loops were used. The results obtained are indicated in Table V.

TABLE V

Absorption of sodium iodide from successive loops. The columns headed A show the percentage of NaI absorbed from each loop. The columns headed B show the total quantity of NaI in grams absorbed before the next loop was injected. The columns headed C show the mean blood pressure in millimeters, corresponding to each loop.

	EXPERIMENT III Dog 3.5 K.			EXPERIMENT VII Cat 2.4 K.		
	A	B	C	A	B	C
First dose.....	57.00	0	80	45.50	0	170
Second dose.....	53.80	0.037	82	10.60	0.041	60
Third dose.....	53.80	0.072	95	33.33	0.046	85
Fourth dose.....	43.00	0.107	95	42.20	0.061	95
Fifth dose.....	27.70	0.135	85	21.70	0.080	95
Sixth dose.....	30.70	0.153	85	33.33	0.090	90
Total NaI absorbed, (grams) ..		0.173			0.105	

TABLE V—CONTINUED

	EXPERIMENT XLVI Cat 4.0 K. (Duplicate loops)			EXPERIMENT XII Cat 3.4 K.		
	A	B	C	A	B	C
First dose.....	40.06	0	140	48.57	0	
Second dose.....	38.07	0.0415	150	48.57	0.034	160
Third dose.....	35.38	0.0809	140	31.42	0.068	
Fourth dose.....	36.00	0.1179	130	27.14	0.090	
Fifth dose.....				14.28	0.109	90
Sixth dose.....						
Total NaI absorbed, (grams)...		0.1553			0.119	

In Experiment III a decline of absorption began after the third dose (that is, in half an hour), the blood pressure running steadily.

In Experiment VII, there was a sharp decline for the second dose, but this is accounted for by the large fall of blood pressure, as will be shown later. Subsequently, the level of absorption and blood pressure recover partly, but incompletely.

In Experiment XLVI, the absorption is but little impaired to the fourth dose, the blood pressure remaining constant.

In Experiment XII, there is a sharp decline after the second dose, the decline increasing to the fifth dose, but there is also a fall of blood pressure which may account for the decline, at least in part.

It appears, therefore, that the administration of iodide into successive loops shows diminished absorption when there is a marked fall of blood pressure. This fall, however, is not constant, and is not due to the iodide, as will be shown later, but to the handling of the intestine.

When the blood pressure remains constant, the absorption does not decline until after three or more doses have been given, and even then the decline is relatively small and within the ordinary variations.

Conclusion: The checking of its own absorption, which is manifested in the loop in which the iodide is placed, does not extend to other loops; these absorb as if no iodide had been given.

Phenol (VIII) gave different results in this connection, the absorption being checked quite early, even without direct contact.

This suggests that the early retardation of iodide absorption cannot be a systemic phenomenon, but must be due to the local contact of the iodide with the mucosa.

7. Stoppage of absorption is not due to the systemic action of sodium iodide

To learn definitely whether any systemic action is concerned in the retarded absorption, I studied the influence of an intravenous injection of iodide on the intestinal absorption.

In Experiment XXXII (dog) two loops, A and AA, acting as controls were injected with 0.01 gram per kilo of sodium iodide (as 1 per cent solution) and excised in ten minutes. Next, 0.08 gram per kilo of sodium iodide as 1 per cent solution was injected intravenously and five minutes afterwards two loops, B and BB, were injected each with 0.01 gram per kilo of sodium iodide (1 per cent) and excised in ten minutes. Loops A and AA absorbed an average of 35.05 per cent and loops B and BB an average of 38.82 per cent.

An increase of 3.77 per cent over that of the control loops was obtained, therefore, in loops after the intravenous injection of sodium iodide when a systemic action might be expected. Hence, neither the presence of a large quantity of iodide in the blood, nor the systemic action of the iodide hinders absorption.

The dose here injected (0.08 gram per kilo) is considerably greater than the amount absorbed in the ordinary experiments. Thus, in the four experiments quoted in Table IV the amount absorbed in ten minutes (when absorption was practically arrested was: 0.0147, 0.0082, 0.0221, and 0.0181 gram per kilo; at most a fourth of that introduced intravenously.

The conclusion that the arrest of absorption is not due to the absorbed iodide is confirmed in the animals in which the absorption from successive loops was measured (see Table V).

In Experiment III, the absorption was not materially reduced until after the fourth dose, the absolute amount absorbed to this time being 0.03 gram per kilo. In Experiment XLVI, there was no material reduction with the fourth dose, although the absolute amount absorbed was

0.04 gram per kilo or three to four times greater than the quantity which is absorbed when the local absorption is checked.

Conclusion: The absorption of iodide is not hindered by the presence of iodide in the circulation; the retardation is therefore not due to the absorbed iodide; it is not due to a systemic action, but is strictly local.

These results are quite different from those obtained with phenol (X). With the latter it was found that the interference is proportional to the amount of absorbed phenol. This is a definite indication that the mechanism is different for the two substances.

8. *Excretion of iodide into the alimentary canal*

We may next consider the possibility that the arrest of absorption might be only apparent, namely, that the iodide might be re-excreted into the alimentary tract and that this re-excretion might just balance the absorption. This could be decided by investigating the iodide excretion into empty intestinal loops. Two observations were made.

Dog XXXVII received a total of 1.2533 grams of sodium iodide into various loops of intestine. At the end of half an hour 0.7715 gram had been absorbed. At this time, the stomach and duodenum, into which no iodide had been injected, yielded 0.0072 gram of iodide, so that 0.93 per cent of the absorbed iodide had been excreted into the stomach and duodenum.

Dog XXXII received

	<i>Gram NaI</i>
At 10:52 intravenously.....	1.2175
From 10:39 to 10:49 absorbed from intestine.....	0.1067
From 11:04 to 11:14 absorbed from intestine.....	0.1182
Total.....	1.4424

At 11:22, half an hour after the intravenous injection, two loops of intestine, which had not been injected with iodide, yielded 0.0067 gram of iodide, or about 0.5 per cent of the quantity injected. At 11:52, one hour after the injection the stomach yielded 0.0269 gram of iodide or about 2 per cent of the quantity injected.

Conclusion: The gastro-intestinal canal excretes a small quantity of the iodide; but the amounts so excreted are very much smaller than those which remain in the intestine when absorption is practically arrested.

For instance in Dog XXXVII, the unabsorbed iodide amounted to 0.4818 gram, the excreted iodide only to 0.0072 gram. The results are even more convincing in Experiment XXXII. In this, the excretion into two loops of intestine during half an hour (from 10:52 to 11:22) after the intravenous injection, was 0.0030 and 0.0037 gram. At 11:04, two similar loops received each 0.1522 gram. Ten minutes later, there was unabsorbed, in one, 0.0818 gram, in the other 0.1044 gram. The excretion could not have been greater than in the empty loops, so that re-excretion would account for at most 4 per cent of the unabsorbed iodide. Re-excretion therefore, cannot be responsible for the arrest of absorption.

This conclusion agrees with that for phenol (V).

9. *The influence of the systemic blood pressure on the absorption*

The arrest of absorption might conceivably be due to changes of the circulation, either of the general blood pressure, or confined to the intestinal vessels. It is impossible *a priori* that the general circulation should have an important share, for it has been shown that arrest of absorption depends upon the local contact with the iodide. However, it seemed worth while to confirm this directly by comparing the absorption with the blood pressure, and to determine what, if any, changes accidental variation of pressure produced in the absorption.

The carotid blood pressure was recorded in all the experiments, and the relation of this to the absorption has been tabulated in the same manner as in our phenol paper (pp. 426-430). Tables were prepared to show the pressures just before injection; the average pressure during the first ten minutes, and the pressure at the end of ten minutes after the injection of iodide respectively. All the experiments ran over ten minutes (the time when absorption is practically completed) and included only loops which were not subjected to experimental conditions other than the injection of iodide.

The absorption was found to be practically constant (53.6 to 65.7 per cent) for blood pressures ranging between 65 and 160 mm. In the one experiment with a "shock" pressure of 35 mm. the absorption was greatly lessened, giving a value of only 30.5 per cent. In the three experiments with pressures between 170 and 190 mm. the absorption was uniformly somewhat below the average (31.52 per cent and 48.50 per cent respectively). This relatively small diminution may be due to the strong vaso-constriction which must have existed in these experiments.

Since absorption is practically completed in ten minutes, it is interesting to compare the results with those obtained after thirty minutes. This may be seen from Table VI. This includes only untreated loops.

TABLE VI

Absorption according to the blood pressure at the end of thirty minutes after injection

BLOOD PRESSURE	AVERAGE PER CENT NAI ABSORBED	NUMBER OF ANIMALS
<i>mm.</i>		
30-40	35.76	3
60-100	49.47	14
110-150	56.30	10
165-180	60.94	2

In these, there is some improvement in absorption with ascending pressures between 60 and 150 mm. but the difference is not very great and falls almost within the ordinary variations.

The relation of blood pressure to absorption is well shown in the experiments with successive doses (Table V) and these lead to the same conclusions:

The absorption was not materially changed by variations of blood pressure between 80 and 95 mm. in Experiment III or 130 and 150 mm. in Experiment XLVI. In Experiment VII, however, a drop of pressure from 170 to 60 mm. diminished absorption from 45.5 to 10.6 per cent. As the pressure recovered to 85 mm. the absorption also improved to 33.3 per cent. In Experiment XII a gradual fall of pressure from 160 mm. to 90 mm. lowered the absorption from 48.6 to 14.3 per cent.

Conclusions: Taking the absorption at the level of 110 to 150 mm. as the normal, there is a slight and somewhat doubtful diminution at pressures between 60 and 100 mm. and a considerable diminution with very low blood pressure (35 mm.), even at this level however, the absorption may be fairly efficient (30 per cent). There also appears to be slightly diminished absorption with excessively high pressures.

These results are nearly the same as with phenol (XII), except that low pressure appeared to interfere even more with phenol. Since phenol itself may lower blood pressure to the shock-level, this constituted one factor in the stoppage of absorption by this drug. Sodium iodide by the intestine, however, does not lower the blood pressure; and therefore the blood pressure is not a factor in explaining the arrested absorption with this drug.

This is seen very plainly in the experiments on "time of sojourn," Table IV. In Experiment II, absorption was completely arrested in half an hour, but the blood pressure continued between 80 and 75 mm. for two hours. In Experiment IV, absorption was arrested in ten minutes; blood pressure had fallen from 195 to 180 mm. In Experiment XIV, the absorption was temporarily arrested in ten minutes with pressure unchanged at 120 mm. In Experiment XVI, absorption was temporarily arrested in ten minutes; pressure unchanged at 140 mm.

10. The influence of local vasomotor drugs on the absorption

During life the cardiovascular mechanism doubtless plays the most important part in the absorption and distribution of most substances which come within reach of it. If this mechanism is deranged or altered in any way, especially in the absorbing area, it would be tempting to explain the variation on this basis. It has been frequently emphasized, however, that the condition of the general blood pressure cannot give any adequate idea of circulatory changes in a given organ. It was, therefore, necessary to test the matter by the direct application of various agents into the lumina of duplicate loops of intestine. After a sojourn of ten minutes, ordinarily, the iodide was injected and allowed to remain with the drug for half an hour using duplicate loops as controls. Sodium nitrite and calcium chloride were removed by washing before the iodide was injected.

Before entering upon the discussion of the data, something must be said as to the visible response of the intestine towards the various measures used. Only gross differences were ascertained by direct inspection. Ice blanched the loops and lowered the local temperature. The tearing of mesenteric nerves brought about no visible difference from normal and the same can be said of epinephrin, strophanthus, chloral, calcium chloride and digitalis. After croton oil and mustard the intestine contained large quantities of serum. Formaldehyde blanched and intensely hardened the loops. Sodium nitrite gave the mucosa and the serosa each a brown aspect owing to the formation of methemoglobin which was also indicated elsewhere throughout the animal's body. This lasted throughout the experiment, but the blood pressure was maintained at a high level. Pinching the loops produced a bright red color, i.e., marked hyperemia.

The results of these experiments are presented in Table VII. All other influences such as shock and low blood pressure have been excluded.

TABLE VII

Effect of local vasomotor changes on the absorption of sodium iodide

LOCAL TREATMENT	AVERAGE PER CENT SODIUM IODIDE ABSORBED FROM DUPLICATE LOOPS		DIFFERENCE IN AVERAGE PER CENT BETWEEN THE TWO LOOPS + INCREASED - DECREASED	NUMBER OF LOOPS ABSORPTION HINDERED: IMPROVED
	Treated loops	Untreated loops		
Group I				
Croton oil.....	9.64	52.55	+17.09	0:4
Intestine pinched.....	74.10	61.89	+12.21	0:2
Spirits of mustard.....	59.14	52.55	+ 7.09	1:3
Formaldehyde 40 per cent...	20.39	34.00	-13.61	4:0
Ice to peritoneal surface of loop.....	59.69	69.38	- 9.69	2:0
Group II				
Sodium nitrite.....	57.62	75.57	-17.95	4:0
Calcium chloride.....	68.05	75.57	- 7.52	2:2
Chloral 10 per cent.....	60.46	61.33	- 0.87	2:2
Mesenteric nerves of loop torn	63.33	55.29	+ 8.04	3:3
Tincture digitalis.....	69.07	61.89	+ 7.18	0:2
Tincture strophanthus.....	58.97	52.55	+ 6.42	1:3
Epinephrin 1 to 1000.....	60.25	55.27	+ 4.96	2:4

The quantitative results as shown in Table VII appear somewhat involved. I have arranged them in two groups to facilitate comparison. The first group consists of those measures which produced a visible reaction on the intestine and are arranged in order of greatest efficiency in promoting or inhibiting absorption. The second group contains those measures which did not alter the intestine visibly and are also arranged in order of greatest efficiency as promoting or inhibiting absorption.

Turning to the measures which produced a visible reaction (Group I) it will be seen that in this series all the measures which produced hyperemia also increased the absorption, the two phenomena tending to go parallel, i.e., both the hyperemia and absorption were greatest with croton oil, least with mustard; pinching being intermediate. Whether this parallelism is a mere coincidence or whether the increased absorption can be attributed to the hyperemia is difficult to say.

The results obtained on normal animals by different investigators are rather contradictory, but it is doubtful whether they were working with simple hyperemia. This has been notably observed for quassia by Brandl⁸ and Rieder.⁹ Brandl working with fistula dogs found that quassia inhibited the absorption of sugar and peptone entirely and that it reduced the absorption of sodium iodide from 10 per cent to 4.3 per cent. Rieder observed in man a tendency to diminished excretion of potassium iodide after quassia, also a prolonged excretion of salicylic acid after taking phenyl salicylate and in dogs it delayed the appearance of strychnine convulsions. These phenomena were attributed by Rieder to a diminished absorption. Other forms of hyperemia such as that resulting from the application of hot air to the skin produced an increased absorption of lactose in frogs and man according to Klapp.¹⁰

On the other hand, Jodlbauer¹¹ working with a Vella fistula in the small intestine of dogs noted no change in absorption of 1 per cent glucose and sodium chloride not only after quassia, but also after hops, absinthin, quinine sulphate and oil of mustard when the drug was introduced with the glucose or sodium chloride. But, if the measure was

⁸ Brandl: *Z. f. Biologie*, 1892, xxix, p. 277.

⁹ Rieder: *Arch. f. exp. Path. u. Pharm.*, 1910, lxiii, p. 305.

¹⁰ Klapp: *Arch. f. exp. Path. u. Pharm.*, 1902, xlvii, p. 86.

¹¹ Jodlbauer: *Arch. intern. de pharmacodynamie*, 1902, x, p. 201.

given one hour previously, there was an increase in absorption. Brandl states that the quassia rendered the intestine hyperemic, but he does not state whether the hyperemia was greater than that produced by the iodide alone. In any case, the action of quassia is probably not that of a simple hyperemia so that the results may have no direct bearing on the present problem. It must also be remembered that absorption from the intact alimentary tract is more complex and involves more unknown factors than absorption from a ligated loop of intestine.

The remaining measures of Group I, that is, formaldehyde and ice each produced a marked reaction. Formaldehyde is also a necrotic. This will be referred to in the next section. In both instances absorption was inhibited. The results with ice seemed to support the old conclusion¹² that cold hinders absorption.

In the group of measures which produced no visible reaction the results are less satisfactory. For instance, the results after digitalis and strophanthus are entirely unexplainable on the basis of a vaso-constriction. In each case there was increased absorption when the contrary would have been expected. Perhaps these two agents acted as irritants with the production of a hyperemia and in this way were responsible for the increased absorption. The action of epinephrin is brief and, in fact, a local vaso-constriction may be followed by a vaso-dilatation. At any rate, the results obtained cannot speak for vaso-constriction. Results with chloral, calcium chloride, and tearing of nerves were entirely unsatisfactory if judged by the response of the loops although the tendency of the average percentage of absorption appeared to be somewhat lessened for calcium chloride and increased for tearing of nerves, but remained unchanged for chloral. Just what happened after sodium nitrite is also difficult to conjecture. If the local mass movement of blood was increased, an increase in absorption would have been expected. The action of sodium nitrite is again comparatively brief, but for the ordinary expected action the results are conflicting.

In one experiment (XLVII), in which all other conditions were

¹² Claude Bernard, cit. Kossa: *Arch. f. exp. Path. u. Pharm.*, 1895, xxxvi, p. 120; Brunton and Cash, cit. Lauder Brunton; *Pharmacology, Materia Medica and Therapeutics*, London, 1885; Kossa, loc. cit.; Klapp, loc. cit.

constant and normal the iodide was introduced into loops of equal length together with the drugs, using one untreated loop as a control. After a sojourn of half an hour, the loops were excised and the absorption determined. In this way the action of the vasomotor agent would certainly occur during the absorption of the iodide.

The results obtained were as follows (figures after each drug indicating the percentage of absorption):

Control—34.06 per cent. *Doubtful Effect*—Digitalis, 33.33; sodium nitrite, 33.33; epinephrin, 29.78. *Diminished absorption*—Caffein, 25.96; calcium chloride, 25.96; strophanthus, 22.40.

Conclusion: Visible hyperemia increases and visible anemia diminishes, the absorption of iodide, but when drugs with a supposed vasomotor action did not produce a *visible* reaction, then the absorption was unchanged.

On the other hand, injury to the epithelium retards absorption markedly:

11. *Absorption is diminished by necrotic changes in the intestinal mucosa*

This was tested by the application of various corrosives, coagulants and protoplasmic poisons to the mucosa of duplicate ligated loops of intestine. After the poison had been left in the loop ten minutes, the intestine was washed with 0.9 per cent sodium chloride. The iodide was then injected and left for half an hour when its absorption was compared with that from duplicate control loops in the same animal. In Table VIII, which contains the results of these experiments, all other influences such as shock and low blood pressure have been excluded.

An inspection of Table VIII shows at once that the absorption of sodium iodide was diminished after each local treatment instituted. The degree of visible reaction, as well as the average percentage of absorption, varied with the various drugs. The first six measures of the table which produced marked visible reactions all diminished the absorption considerably, the difference ranging from 28.72 per cent to 8.88 per cent. The inhibitory effect of

TABLE VIII

Effect of necrotic changes on the absorption of sodium iodide

LOCAL TREATMENT	AVERAGE PER CENT SODIUM IODIDE ABSORBED FROM DUPLICATE LOOPS		DIFFERENCE IN AVERAGE PER CENT BETWEEN TWO LOOPS + INCREASED - DECREASED	NUMBER OF LOOPS ABSORPTION HINDERED: IMPROVED
	Treated loops	Untreated loops		
Formaldehyde, 40 per cent..	20.39	34.00	-13.61	4:0
Sodium hydroxide 20 per cent.....	30.11	58.83	-28.72	4:0
Sulphuric acid, 50 per cent..	45.17	58.83	-13.66	2:2
Mercuric chloride 1 to 1000.	53.35	73.26	-19.91	2:0
Barium chloride, 1 to 100....	62.45	73.26	-10.81	2:0
Phenol (concentrated).....	71.55	80.43	- 8.88	2:0
Crushing of loop.....	50.77	60.77	-10.00	2:0
Alcohol, 95 per cent.....	20.40	34.00	-13.60	4:0
Chloroform, 10 per cent in 95 per cent alcohol.....	27.39	37.45	-10.06	2:0
Chloroform, 100 per cent....	56.39	63.39	- 7.00	1:1
Sodium Fluoride, 1 per cent	47.60	73.15	-25.55	2:0

phenol confirmed our former observation.¹³ Crushing of the loops by compression between the prongs of a pair of forceps produced multiple punctate hemorrhages on the serous and mucous surfaces with desquamation of the epithelium of the mucosa and oozing of serum. In other words it injured very severely the absorbing surface of the intestine. The results show an impairment in the function of absorption for sodium iodide. Alcohol and chloroform produced no marked visible reaction. After alcohol the intestine seemed warmer to the touch than the control. In both instances absorption was diminished. As far as alcohol is concerned my observations are not in accord with those of Brandl¹⁴ on fistula dogs. This investigator found alcohol to increase the absorption of sodium iodide, sugar and chloral five fold. Brandl's alcohol was a 20 per cent solution while that used by me was 95 per cent and the experiments in each case were conducted somewhat differently. Chloroform in alcohol seemed to inhibit absorption better than chloroform

¹³ Sollmann, Hanzlik and Pilcher: loc. cit.¹⁴ Brandl, J.: loc. cit.

alone, namely by 10.06 per cent for the former against 7.00 per cent for the latter. Sodium fluoride was chosen as a direct protoplasmic poison. No gross effect was visible but absorption was diminished by 25.55 per cent—a very pronounced inhibition.

As judged from the average percentage of absorption, and the number of loops involved the various agents arrange themselves in order of greatest efficiency in diminishing absorption as follows—sodium hydroxide, sodium fluoride, mercuric chloride, formaldehyde, alcohol, barium chloride, chloroform in alcohol, crushing the loop and phenol. Sulphuric acid and chloroform occupy an indefinite position.

Conclusion: Injury of the intestinal epithelium however produced, hinders the absorption of sodium iodide.

This result differs from that obtained with phenol (VII c, d, and e) of which the absorption was not retarded by formaldehyde and fluoride, although alcohol and phenol itself had a retarding effect. This again speaks for the importance of the epithelium in the absorption of iodide.

12. The specific local effect of halogens on the absorption of iodide

The experiments so far described have shown that the inhibition of the absorption of iodide is not due to a systemic action, but is a local effect. They have further shown that the absorption is not influenced by such moderate changes in the intestinal circulation as the iodide might be supposed to produce; but that the absorption is hindered materially by chemical changes in the absorbing epithelium. This suggests that the mechanism by which iodide inhibits its own absorption might be something similar; that the iodide might also produce some chemical change in the absorbing epithelium, perhaps by “saturating” an affinity of the epithelium for free halogen ions, which might be an essential condition for absorption. To test this working hypothesis, experiments were made on living and excised intestine. The results on the intact animal conform to this view. Those on the excised intestine were negative; but since they present some interesting features of their own, they may be discussed here and thus disposed of.

13. *The excised intestine is slowly permeable to sodium iodide*

The suggested hypothesis of a diminished local permeability due to an increased concentration of sodium iodide in the intestinal wall was first tested by studying the rate of diffusion of sodium iodide, inclosed in a loop of excised intestine, which was suspended in Locke's solution. According to the hypothesis, it was supposed that the rate of diffusion would become progressively slower, as the intestinal mucosa becomes "saturated" with the iodide.

A recently excised loop of intestine of about 15 cm., length was filled with a 1 per cent solution of sodium iodide in water and immersed in a beaker containing 200 cc. of Locke's solution (without glucose) kept at 37° C. Both ends of the loops were closed conveniently by tying the stems of separating funnels into the open ends. The stopcocks could then be closed or opened at will and more iodide introduced without handling the loop. At the beginning of each period, the iodide solution in the loop was thus replaced with fresh iodide solution by manipulating the separating funnel. A fresh beaker of Locke's solution was substituted and the iodide which had diffused into the first Locke's solution was estimated. This was repeated for several periods (five).

The periods were at first spaced at five, then at fifteen minutes; but even this was too short, the quantity of perfused iodide being too small for quantitative estimation. One hour periods gave satisfactory results. In three such experiments, the rate of diffusion increased instead of diminishing as was expected. This was shown graphically in Figures II and III which were constructed from the results of Experiments XXXVII, XXXIX and XLI.

This result, although unexpected, does not disprove the hypothesis for the living animals; for the conditions may differ in the two cases in several important respects. For one thing, and perhaps the most important, the diffusion through the excised intestine involves *all* of its coats and not only the epithelium as in life. Moreover, the epithelium itself may functionate somewhat different in the excised intestine.

In connection with the study of the behavior of the excised intestine, Dr. Sollmann called my attention to a statement of

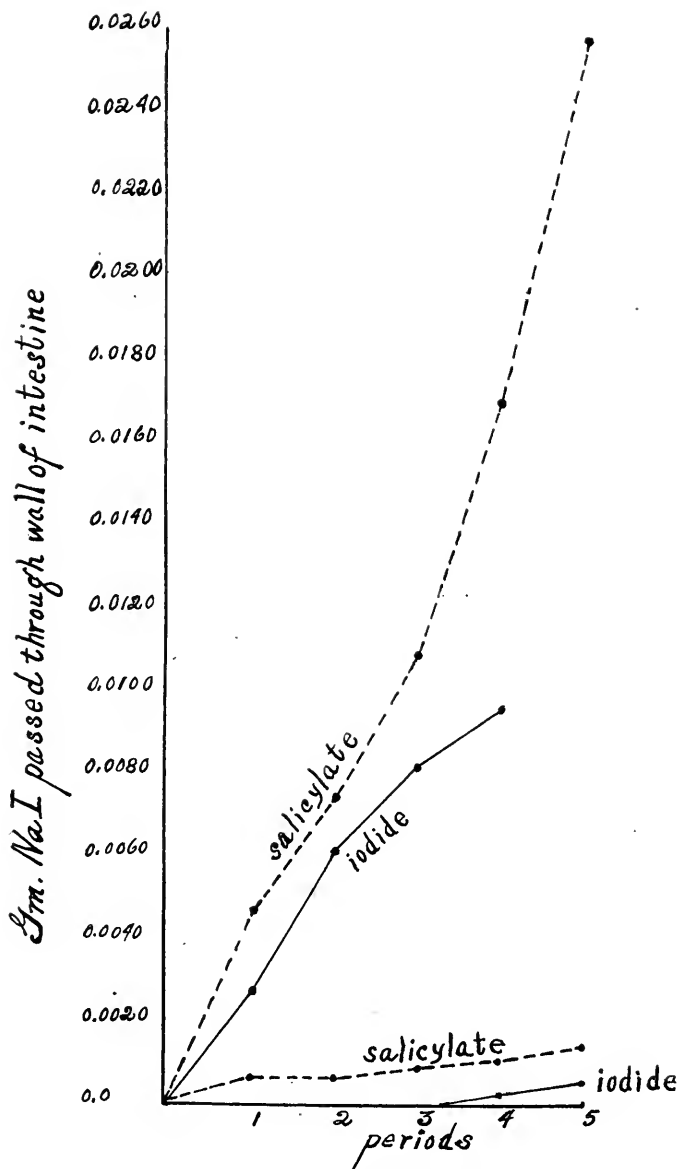


FIG. II. THE PERMEABILITY OF SODIUM IODIDE THROUGH THE EXCISED INTESTINE AND THE EFFECT OF SODIUM SALICYLATE ON THE PERMEABILITY

The solid lines represent 1 per cent sodium iodide; and the broken lines 1 per cent sodium salicylate containing 1 per cent of sodium iodide. The two lower curves denote periods of half an hour duration; the two upper curves of one hour duration.

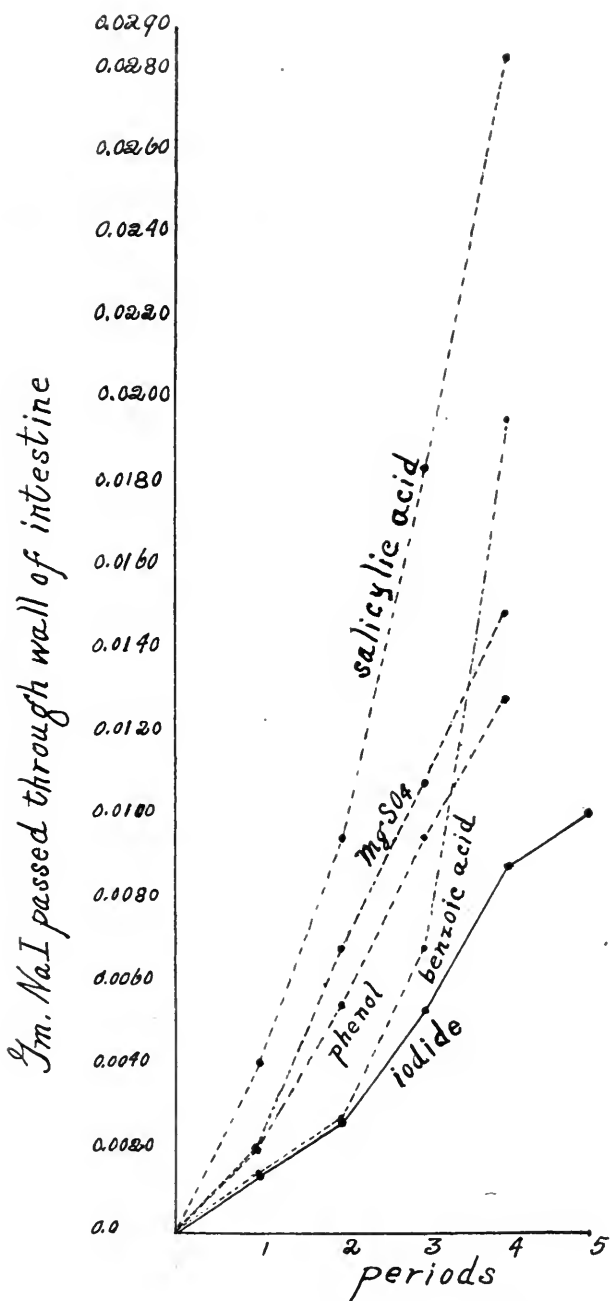


FIG. III. THE EFFECT OF VARIOUS SUBSTANCES ON THE PERMEABILITY OF SODIUM IODIDE THROUGH THE EXCISED INTESTINE

The solid line represents 1 per cent sodium iodide alone. The broken lines represent the various substances containing 1 per cent of sodium iodide as follows; salicylic acid 0.3 per cent; benzoic acid (saturated solution); phenol 1 per cent, magnesium sulphate 5 per cent. All the periods are of one hour duration.

Oswald's concerning the permeability of colloids.¹⁵ Oswald states that salicylic acid increases the diffusion of electrolytes through colloids and suggests that electrolytes might do likewise. It seemed interesting to try this with excised intestine. This was done in the following manner. A solution of 1 per cent sodium iodide was made in aqueous solutions of the following substances; phenol 1 per cent, salicylic acid 0.3 per cent, sodium salicylate 2 per cent, benzoic acid (saturated solution), and magnesium sulphate 5 per cent. The respective solutions were placed into separate loops using a control loop containing sodium iodide alone. In all other respects the experiments were conducted as for the permeability of sodium iodide as already described. The results appear in the form of curves in Figs. II and III.

It is plainly evident in these curves that all of the substances used, but especially salicylic and benzoic acid, increased the permeability to sodium iodide. I am thus able to confirm the statement of Oswald's concerning colloid permeability in this direction. I have not sought for an explanation of the phenomenon.

Conclusion: Sodium iodide does not easily diffuse through the excised intestine. The rapidity of perfusion increases progressively, for at least five hours. Certain substances such as salicylic acid, sodium salicylate, benzoic acid, phenol and magnesium sulphate increased the permeability.

In connection with this, reference may be made to certain other experiments which also do not bear directly on the solution of the point in question. I refer to the following section.

14. (a) *Post-mortem absorption of iodide*

With phenol (VIIIf) post mortem absorption was considerable and varied from 2 per cent to 47.5 per cent with an average of 16 per cent. The absorption of sodium iodide after death was also considerable but more constant and gave higher averages than of phenol. In one animal three loops of equal length

¹⁵ Oswald, A.: *Zeit. f. exp. Path. u. Therap.*, 1910, viii, p. 226. In a private communication, Dr. Oswald informs me that he can not, at present, locate the original source of his information.

absorbed in a half hour 40.71 per cent, 28.88 per cent, 33.26 per cent respectively, with an average of 34.28 per cent. In two other animals the absorption from the stomachs was 33.91 per cent and 37.76 per cent respectively, with an average of 35.83 per cent.

(b) The distribution of the unabsorbed iodide between the intestinal wall and contents

In the case of phenol (XVI) a considerable amount of the substance was found to be retained in the wall of the intestine during life. Sodium Iodide was examined in the same way.

The viscus was rinsed and these washings with the contents form column A. The wall was then macerated in water for an hour, the filtrate forming column B. The wall was again macerated with fresh water for further three hours (C), and then again for further twenty hours (D). This thoroughly extracted tissue forms the material for column E. The figures present the percentage of the total unabsorbed iodide which is contained in each of the extracts.

The results recorded in Table IX show that the iodide behaved like phenol.

TABLE IX

Distribution of unabsorbed iodide

PERCENTAGE OF IODIDE RETAINED IN	A CONTENTS WITH SLIGHT RINSING	B MACERATE OF ONE HOUR	C MACERATE OF THREE HOURS	D MACERATE OF TWENTY HOURS	E WALL OF VISCUS	TOTAL IODIDE ABSORBED
54b—Loop of intestine for half hour during life.....	79.02	6.90	1.73	3.44	8.76	50.00
54c—Loop of intestine for one hour during life.....	36.43	13.64	4.57	9.07	36.29	81.17

15. The local inhibitory effect of haloids on the absorption of iodide from the intestines of living animals

In the preceding sections it was shown that the inhibition of absorption is a local effect (section 6, "successive doses"); that it cannot be explained by changes in the general or local circu-

lation (sections 9 and 10); but that injury to the epithelium greatly retarded absorption (section 11). This prompted the working hypothesis that the inhibitory action of iodide might be due to some specific modification, possibly a saturation of this epithelium. This could not be confirmed in the excised intestine and it was therefore necessary to modify the experiment so as to make it applicable to the living animal.

For this purpose, I took advantage of the well known similarity in the absorption, diffusion and excretion of chlorides and iodides. If the working hypothesis were correct, then the absorption of iodide should be checked by the application of sodium chloride to the intestine. Since small quantities of dilute ("physiological") solutions of sodium chloride, placed in the intestine, have no effects on the general and local circulation, and would not cause a general injury of the epithelium, a positive result would also show that the retarding action must be a highly specific effect on the epithelium, in consonance with our working hypothesis. Two experimental dispositions were adapted to test this theory. In the first series, the duplicate loops were treated with 1 per cent sodium chloride before the iodide was introduced, and the absorption from these loops was compared with the absorption from untreated loops. It was found that *the loops which had been treated with 1 per cent sodium chloride absorbed much less iodide*—in other words, the sodium chloride had inhibited the iodide absorption.

The experiments were conducted as follows: Two loops of intestine of equal length were filled with 1 per cent sodium chloride solution and allowed to remain for fifteen minutes. The chloride was then removed and 10 cc. per kilo of 1 per cent sodium iodide was introduced. Two untreated loops acting as controls were also injected with the same quantity of iodide. At the end of ten minutes all the loops were excised and the iodide absorption was determined in the usual manner. The following results were obtained:

Experiment XLIX

	<i>Per cent NaI absorbed</i>
Untreated loop B.....	26.47
Untreated loop BB.....	28.88
Average.....	<hr/> 27.68
NaCl treated loop A.....	3.72
NaCl treated loop AA.....	3.72
Average.....	<hr/> 3.72

The average blood pressure was comparatively low and somewhat variable, otherwise the animal was in good condition.

Experiment LI

	<i>Per cent NaI absorbed</i>
Untreated loop B.....	44.54
Untreated loop BB.....	48.08
Average.....	<hr/> 46.31
NaCl treated loop A.....	25.95
NaCl treated loop AA.....	25.95
Average.....	<hr/> 25.95

The average blood pressure was high and constant throughout the experiment, the general condition of the animal was good.

These two experiments show definitely that there was an inhibition of absorption of the iodide in loops which had been treated previously with 1 per cent sodium chloride. For instance, in Experiment XLIX, absorption was diminished about eight times, and in Experiment LI about twice, as compared with the control loops.

The inhibition in each case took place in spite of the difference in the systemic blood pressures—which in one case was low and variable and in the other high and constant.

Conclusion: The application of sodium chloride to the intestinal mucosa causes an inhibition of the absorption of sodium iodide.

A second series of experiments was aimed to determine whether the simultaneous presence of chloride and iodide would influence the absorption. For this purpose, I compared the absorption from a 1 per cent sodium iodide solution with the absorption from a solution containing equal volumes of 1 per cent sodium iodide

and of isotonic (0.4 per cent) sodium chloride. In Section 4, it was shown that the absorption is but little influenced by concentration. The iodide of the mixed solution should therefore be absorbed as readily as that of the undiluted solution. In fact, however, it was found that a much smaller percentage of iodide was absorbed from the mixed solution so that the *presence of the chloride has interfered with the absorption of the iodide*. It is especially significant that this result was produced even by the very low concentration (0.2 per cent) of sodium chloride, which has no other known effect on cellular functions in mammals. We may fairly assume that the chloride has simply taken the place of an approximately equivalent amount of iodide and has therefore inhibited the absorption in the same way as it would be inhibited by an equivalent quantity of iodide.

The experiments were conducted as follows: In Experiment XLVIII, one set of duplicate loops was injected with 1 per cent sodium iodide, a second set with 0.4 per cent sodium chloride, a third set with a mixture of 0.5 per cent sodium iodide and 0.2 per cent sodium chloride solutions. In each case 10 cc. per kilo of the solution or mixture was used. All loops were excised in ten minutes after the injections were completed and the iodide absorption was determined in the usual manner. A fourth set of loops was excised as controls in order to gain an idea of their sodium chloride content (using the Volhard method). In Experiment LI, the absorption of sodium iodide from a set of duplicate loops injected with 1 per cent sodium iodide solution was compared with that of another set of duplicate loops which were injected with 0.5 per cent sodium iodide and 0.2 per cent sodium chloride. The results were as follows:

LOOP	SOLUTION	PER CENT SODIUM IODIDE ABSORBED	
		Experiment XLVIII	Experiment LI
A.....	0.5% Na+0.2% NaCl	11.98 } 10.96	33.77 } 33.77
AA.....	0.5% Na+0.2% NaCl	9.95 } (average)	33.77 } (average)
B.....	1% NaI	34.79 } 34.03	44.54 } 46.31
BB.....	1% NaI	33.26 } (average)	48.08 } (average)
C.....	0.4% NaCl	trace	
CC.....	0.4% NaCl	trace	

It was very probable that the iodide also inhibits the absorption of chloride, but this could not be proven, since the presence of chloride in the normal intestine introduced too great an experimental error.

16. *The effect of intravenous injection of sodium chloride on the absorption of iodide*

It was shown in the preceding section that the absorption of iodide is inhibited by the local contact of a haloid salt with the intestinal surface. It appeared interesting to learn whether this modification in the absorbing power of the epithelium could be produced otherwise than by direct local contact, namely by increasing or diminishing the haloid store of the body at large. Intravenous injections were made to increase the haloid store without local contact. In Section 7, it was shown that the intravenous injection of sodium iodide does not inhibit the absorption acutely. In this experiment (XXXII), the intravenous injection preceded the local application of the iodide by only five minutes, and this time might be too short for the passage of the iodide from the blood into the intestinal epithelium, and for the saturation of the latter. In the present series, therefore, I separated the intravenous injection of sodium chloride and the local application of iodide by longer intervals (from one hour to two days), and then determined the curve of absorption during two hours.

The experiments were conducted as follows: Intravenous injections of 0.9 per cent sodium chloride, 100 cc. per kilo, were given to each of five dogs.

Dog. LII was used one hour after the injection. His condition remained practically the same as before the injection.

Dog LIII was used half a day afterwards. He appeared very sick, developed an oedema of the leg corresponding to the site of injection and upon opening the abdomen the intestine appeared water logged, thick and unusually large in diameter and had a purplish hue, which, however, returned to a normal red during the course of the experiment. The peritoneal cavity contained considerable fluid. The blood pressure throughout the experiment was good. This dog urinated considerably about six hours after the injection.

Dog LIV was used one day after the injection. He appeared composed and suffered no inconvenience, developed no oedema of the limbs, and the intestines appeared no different from normal. No history as to urination was available. His blood pressure was good.

Dog LV was used one day after the injection. He appeared weak but was able to stand up; intestinal wall was thick and diameter large; no fluid in peritoneal cavity; urinated about 1000 cc. during twenty-four hours; blood pressure was good.

Dog LVI was used two days after the injection. He seemed cheerful, ate some bread on the second day and drank a little water. The intestinal wall seemed thicker than usual and somewhat boggy; peritoneum was normal; blood pressure was good, but towards the end of the experiment, the animal developed pulmonary oedema.

After the stated intervals the dogs were anesthetized and the absorption of iodide was determined according to "time of sojourn." That is, equal quantities of 1 per cent sodium iodide (10 cc. per kilo) were introduced into duplicate intestinal loops of equal length and these were excised at successive intervals of ten minutes, half hour, one hour and two hours.

The results of the individual experiments as shown in Fig. IV, are fairly similar with the single exception of Experiment LIV. We are therefore justified in using the average curve for comparison.

On comparing the average curve of the hyperchloric animals with the average curve of the normal or chloride-poor animals (Fig. V) it will be seen that the absorption in the first ten minutes is considerably (and fairly constantly) lower in the salted animals than that of the normal animals. This might be interpreted as an inhibitory effect; but it may be only accidental, for the difference disappears within half an hour, and thereafter the salted animals appear to absorb considerably more than the normal animals. We cannot, therefore, affirm that the absorption of iodide is inhibited by rendering the tissues at large rich in haloids. The inhibition appears to require direct local contact with the haloid.

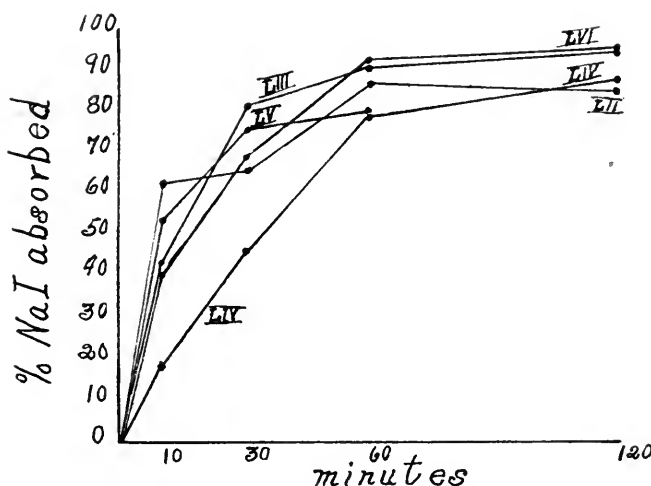


FIG. IV. ABSORPTION OF SODIUM IODIDE ACCORDING TO TIME OF SOJOURN IN HYPERCHLORIC ANIMALS

Experiment LII—One hour interval before the iodide injection.
 Experiment LIII—Half day interval before the iodide injection.
 Experiment LIV—One day interval before the iodide injection.
 Experiment LV—One day interval before the iodide injection.
 Experiment LVI—Two days interval before the iodide injection.

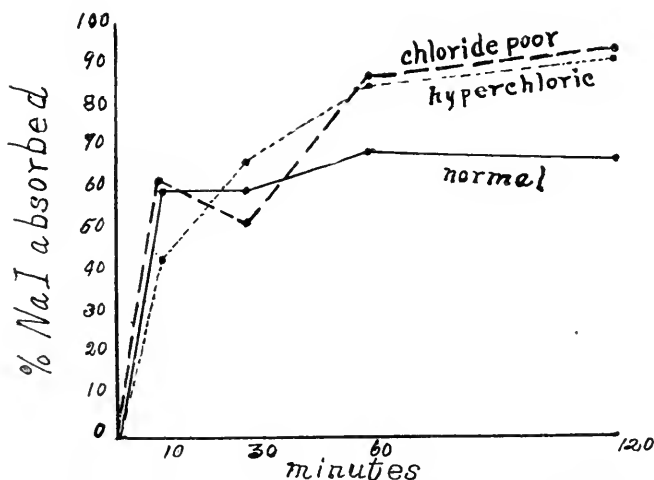


FIG. V. AVERAGE CURVES OF ABSORPTION OF IODIDE IN NORMAL, CHLORINE-POOR AND HYPERCHLORIC ANIMALS

----- Mean curve of absorption for hyperchloric animals (five dogs).
 - - - - - Mean curve of absorption for chloride-poor animals (three dogs).
 ————— Mean curve of absorption for normal animals (four dogs).

17. *The absorption of iodide in chloride-poor animals*

It seemed worth investigating whether a reduction in the chloride store of the organism would influence the iodide absorption. Conceivably this might occur through a greater avidity of the haloid starved tissues, or through a lesser preliminary saturation of the intestinal epithelium. To test the matter, the course of absorption was studied as in the previous section, but on dogs which had been fed on chloride-poor food.

The experiments were performed in the following manner:

Three dogs were placed on a chloride-poor diet consisting of water and milk. At first, each dog was given 500 cc. of milk per twenty-four hours and a liberal quantity of water. Later on, in about the middle of the period, the milk was reduced to 250 cc. and this was maintained to the end until the animals were used. The liberal quantity of water was kept up throughout all periods. The excretion of chlorides was observed qualitatively by testing the urine periodically with silver nitrate and nitric acid. In the beginning of the experiments, before the dogs were placed on the chloride-poor diet all urines exhibited copious quantities of chlorides. In no case did the urine become chloride free. Dog XXIX having been kept on the diet eighteen days lost 1.6 kilos in body weight and became so emaciated and weak that it was used for the absorption experiment at the end of this time. The urine of this dog still showed a marked trace of chlorides. Dog XXX lost 1.0 kilo in weight at the end of twenty-five days, was emaciated and almost unable to stand up. The urine in this case still showed a trace of chlorides. Dog XXXI lost 1.5 kilo in weight in twenty-seven days. He appeared cheerful and his general condition was good. The urine still showed a trace of chlorides.

The chloride excretion was not observed quantitatively inasmuch as this would not have added materially to our knowledge concerning the chloride content of the tissues. I contented myself with placing the animals upon the customary chloride-poor diet employed routinely in laboratories for this purpose. The experiments were conducted over a much longer period than is usually required to diminish the chloride excretion to its minimum. In two instances, at least, the animals were kept on the diet up to time when indications of intoxication were beginning to be manifest. On the basis of observations previously made by others it may be fairly considered that these dogs were rendered practically as chloride-poor as was possible.

The results are shown in Fig. VI. They are practically parallel, so that the average curves shown in Fig. V, may be used for comparison. It will be seen that the absorption during the first ten minutes agrees quantitatively with that of the normal animals and is considerably greater than that for the chloride-rich animals. After an hour, the absorption is materially greater than in normal animals, but no greater than with chloride-rich animals. The data are therefore equivocal. The absorption is good, but the difference is not sufficiently large to justify conclusions. It is not permissible to say that the deprivation of chlorine has any material effect. This again confirms the conclusion that the inhibitory effect of haloids is practically entirely local.

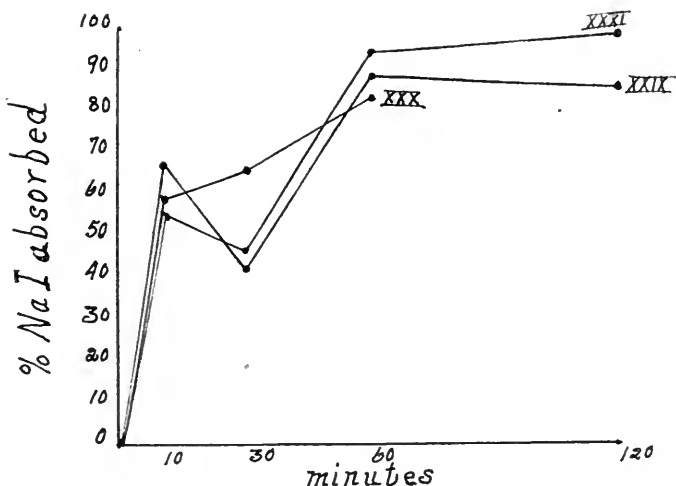


FIG. VI. ABSORPTION OF SODIUM IODIDE BY ANIMALS WHOSE TISSUES WERE POOR IN CHLORIDES.

SUMMARY

(The numbers correspond to section numbers in the text.)

1. The quantitative absorption of sodium iodide from the gastrointestinal tract is practically the same in dogs and in cats.

2. The average absorption is approximately the same for all divisions of the small intestine; from the stomach and colon it is about a third less.

3. The extent of surface does not influence the percentage of absorption.

4. One, 2 and 5 per cent solutions of sodium iodide are absorbed with about the same ease; a 10 per cent solution somewhat more readily.

5. The absorption from the intestine is at first very rapid so that 50 per cent to 75 per cent are absorbed within ten minutes. By that time the absorption is greatly checked or completely arrested so that 25 per cent to 50 per cent may remain unabsorbed at the end of two hours.

6. The checking of the absorption in a loop containing iodide does not affect absorption in other loops.

7. Stoppage of absorption is not due to the systemic action of sodium iodide.

8. Re-excretion is not responsible for the arrest of absorption.

9. In general there is practically no relation between the absorption and variations in blood pressure unless the pressure reaches a very low level (35 mm.) when the absorption is diminished, but it may be fairly efficient even at this level.

10. Visible hyperemia increases and visible anemia diminishes the absorption of iodide.

11. Injury of the intestinal epithelium, however produced, hinders the absorption.

12. The inhibition of absorption of sodium iodide is a local effect; the explanation must be sought in some chemical change in the absorbing epithelium.

13. Sodium iodide does not easily permeate the excised intestine. Certain substances increase the permeability.

14A. Post-mortem absorption of iodide is considerable and quite constant.

B. A considerable amount of the iodide is retained in the wall of the intestine during life.

15. *The application of dilute (0.2 to 1 per cent) sodium chloride to the intestinal mucosa inhibits the absorption of sodium iodide.*

16. It is doubtful whether absorption of iodide is inhibited by rendering the tissues at large rich in haloid.

17. It is doubtful whether the absorption of iodide is materially increased by rendering the tissues poor in chlorides.

The course of the absorption of iodide is superficially very similar to that of phenol. Both show a rapid initial absorption, succeeded by a marked inhibition of absorption. The mechanism of this absorption, however, is entirely different in the two cases. With phenol, the inhibition is due to an interference with the circulation, which may be brought about by systemic or local application. With iodide, the effect is directly on the absorbing epithelium, and occurs only on direct contact with a haloid salt. It is well known that contact with haloids also alters the permeability of the kidney cells to haloids.¹⁶ The two facts are presumably related.

I wish to express my thanks to Professor Sollmann for his guidance in this research; for his help in criticising the results and conclusions, and in preparing the manuscript.

¹⁶ Sollmann: *Am. J. Physiology*, 1903, ix, p. 426.



A STUDY OF THE ACTION OF VARIOUS DIURETICS IN URANIUM NEPHRITIS¹

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Synopsis.....	423
Introduction.....	423
Review of literature.....	425
Discussion of the technique employed in the experiments.....	426
Course of the experiments.....	429
The effect of diuretics in uranium nephritis.....	430
The renal pathology.....	433
Summary.....	435
Bibliography.....	437

In a recent anatomical study (1) of the nephritis produced in the dog by the use of various nephrotoxic substances it has been shown that these substances vary to some extent in the degree of their selective affinity for the different kidney tissues. Arsenic, for example, has a striking affinity for the blood vessel tissue of the kidney; while potassium dichromate causes an involvement of the epithelial element of the kidney much earlier than does any of the usually employed nephrotoxic substances.

Uranium nitrate, a substance which has frequently been employed to produce experimentally a nephritis, in its avidity for the different tissues of the kidney, is not so selective in its action as are the poisons just mentioned.

If uranium be given in large doses subcutaneously, or if smaller quantities be used and the nephritis be allowed to persist for some days, the nephritis which it induces with such a technique is more tubular than vascular. If, on the other hand, small quan-

¹ Presented in abstract before the Society for Pharmacology and Experimental Therapeutics, Baltimore, December 27, 1911.

tities are employed, 5 to 10 mgs. per animal, and if the nephritis be terminated early, the reaction on the part of the kidney is largely vascular. We possess, therefore, in uranium a nephrotoxic substance, which when appropriately administered is competent to produce the two main types of nephritis. For this reason uranium was the nephrotoxic substance selected to use in the production of nephritides of different severity, in which one or both elements of the kidney concerned in the formation of urine were functioning pathologically.

By the use of such a substance which produces primarily a vascular, and later a tubular nephritis, it was hoped that by studying the physiological response of the kidney at these stages of its pathological reaction, it might be determined which element of the kidney in a nephritis was most concerned in determining the quantitative output of urine. With this object in view in this study diuretics have been employed which effect both the vascular and the epithelial elements of the kidney.

In the anatomical study of experimental nephritis which has been previously referred to, the nephrotoxic substances employed were potassium dichromate, sodium arsenate, cantharidin and uranium nitrate. During the course of this investigation it was noted that there existed a fairly clear cut correlation between the degree of epithelial involvement in a given nephritis and the total output of urine; whereas, on the other hand, no such histological correlation could be made, within certain limitations, between the severity of the vascular pathology and the output of urine. For example, a nephritic animal with a normal urine flow, or a polyuria, would show a vascular reaction which histologically would be similar to the vascular pathology in an anuric animal. The associated epithelial reaction in such stages of a nephritis differed very widely. In the early nephritides with a normal output of urine or a polyuria, the epithelial involvement was slight or absent. In some of the experiments, especially those conducted with uranium, the epithelium appeared to have undergone a shrinkage. In the later stages of the nephritis when the output of urine had been reduced or an anuria had developed, the epithelium and especially that of the convoluted tubules,

invariably showed marked alterations. The epithelial changes varied with the severity of the nephritis. The earlier degenerations consisted in cloudy swelling and vacuolation, while the later changes were principally an epithelial desquamation, usually preceded by necrosis.

In these late nephritides the swelling of the epithelium was frequently decidedly noticeable and was sufficient either greatly to encroach upon or completely occlude the lumen of the tubules.

The present physiological study of the nephritic kidney has been undertaken to determine, if possible, the part played by the vascular and by the epithelial pathology of the kidney in influencing the output of urine, and to determine whether or not the vascular mechanism of the kidney is physiologically responsive in a nephritis in which there is evidence of epithelial involvement and but little histological evidence of vascular injury.

REVIEW OF LITERATURE

The two most important recent contributions to the study of acute experimental nephritis are those by Schlayer (2) and Hedinger and by Pearce (3), Hill and Eisenbrey.

These investigations were conducted with the same general object in view and are principally concerned with the physiological response of the nephritic kidney.

Schlayer and Hedinger studied the vascular reaction of the kidney in both the glomerular and the tubular types of nephritis. For their studies in the vascular type of nephritis they employed as kidney poisons, cantharidin, arsenic and diphtheria toxin, and for the tubular type potassium chromate and corrosive sublimate.

The investigation by Pearce, Hill and Eisenbrey was also principally concerned with the vascular reaction in acute nephritis. The authors were able to distinguish types of nephritis in which either the tubular or the vascular changes predominated. They were not able to conclude, however, that a given poison produced an exclusively tubular or vascular injury. Potassium chromate, corrosive sublimate and uranium nitrate, caused extensive tubular injury and in the early stages of the nephritis showed no evidence

of vascular injury except physiologically. When physiological methods were employed they were able to demonstrate in the early stages of the nephritis an exaggerated contraction and dilatation of the vessels and also an increased diuresis. Arsenic and cantharidin acted as vascular poisons and produced but little injury to the tubules. Both of these poisons tended to cause an anuria which was characterized by minimal contraction and dilatation of the renal vessels and little or no flow of urine. Finally in this investigation two types of late tubular nephritis are described: one anuric and accompanied by gastro-intestinal symptoms; and the other polyuric until the time of anesthesia.

In addition to these two investigations which are principally concerned with the physiological response of the kidney, pathological studies of the kidney in a uranium nephritis have been made by several investigators.

Heineke and Myerstein (4) were able to demonstrate a marked vascular disturbance in the kidney from uranium in addition to a pronounced action on the renal epithelium; while Dickson (5) in an extensive series of experiments in which the guinea pig was the animal employed came to the same conclusions.

Christian (6) in his work on uranium nephritis in which the vascular pathology was studied, described as developing in the capillaries of the glomerulus, oval or irregular homogeneous droplets 0.5 to 4 microns in diameter. Similar structures have been observed in several of the experiments in the series of animals which will be presented in this study.

The work of Schirokauer (7) on the uranium nephritis of rabbits is of special interest on account of the associated anasarca.

DISCUSSION OF THE TECHNIQUE EMPLOYED IN THE EXPERIMENTS

In conducting the experiments the dog was the animal constantly employed. A total of twenty-three animals were used. The animals were free from disease and their general nutrition was apparently normal.

For three days prior to the experiments the animals were kept in metabolism cages, fed on beef and hard bread and given once

a day by stomach tube a known and constant quantity of water. The quantity of water varied with the size of the animals. During the period of preliminary observation the urine was collected daily, measured and studied qualitatively and microscopically. The existence of a naturally acquired nephritis was excluded. Two of the animals showed the presence of albumen and erythrocytes in the urine but no casts.

At the end of three or four days, after the preliminary data had been obtained, the animals were given from 5 to 10 mgs. of uranium nitrate subcutaneously. The frequency with which the injections were repeated was determined by the severity of the nephritis produced by a given injection and by the stage of the nephritis that was desired in which to study the action of the different diuretic substances. Such a method of regulating the quantity of nephrotoxic substance is more accurate, so far as the reaction on the part of the kidney is concerned, than can be obtained by using a constant quantity of the kidney poison per kilogram of body weight, since different animals vary very greatly in their response to the same quantity of the poison.

Usually within twelve to twenty-four hours after the initial injection of uranium the animals had developed a well marked nephritis.

Occasionally on the first day of the nephritis, and almost invariably by the second day, the animals developed a pronounced glycosuria. The quantitative output of albumen was not determined. Quantitative sugar determinations were made with both Fehling's and Purdy's quantitative reagents. These determinations showed that the output of sugar in a twenty-four hour specimen of urine varied from 0.25 to 3.22 per cent.

After the production of the nephritis the animals were anesthetized with either morphine-ether or Gréhan's anesthetic.²

The following operative technique was constantly employed.

A tracheal canula was tied in place and connected with the ether bottle to be used in case additional anesthetic was necessary during the experiment.

²*Gréhan's Anesthetic.* The animal is given $\frac{1}{4}$ cc. per kilogram of a 4 per cent solution of morphine. This is followed in half an hour by 10 cc. per kilogram of the following mixture: Chloroform, 50 cc.; alcohol and water, each 500 cc.

The carotid pressure was recorded in the usual way, and a relative idea of the heart volume was obtained along with the pressure tracing by means of a Hürthle manometer.

The left kidney was surrounded by a rubber bag filled with water, and the kidney with its surrounding water cushion placed in a copper oncometer. The oncometer communicated by means of a rubber tube with a water manometer which registered on an arbitrary scale graduated in millimeters the increase or the decrease in the volume of the organ.

Into each ureter was placed a ureter canula. Observations of the urine flow were made only from the right kidney, on account of the fact that the flow from the left kidney was possibly influenced by the mechanical disturbance necessarily associated with the use of the oncometer.

The various diuretic solutions were given intravenously through the femoral vein, due care being taken of their temperature.

The experiments were of such a nature that they would necessarily require considerable time for consecutive observations of the action of the different diuretics. On this account it seemed advisable to employ some method to maintain a fairly constant body temperature. For this purpose a copper water box was used similar to the ones employed in Sollmann's laboratory. The upper surface of the box is concave and holds a wooden rack in which the animal is placed. With such an apparatus the animal's body temperature can be fairly accurately maintained.

At the termination of the experiments, the kidneys were at once removed and tissue fixed for microscopic study in both corrosive-acetic and in formaline.

Five of the twenty-three animals employed in this investigation were either purposely or accidentally killed before or at the commencement of the anesthetic. Kidney tissue from these animals was fixed for histological study.

In the remaining eighteen animals the physiological response of the nephritic kidney was studied under the influence of:

Caffeine.....	1-2 cc. of a 1 per cent solution per kilogram
Theobromine.....	1-2 cc. of a 1 per cent solution per kilogram
Digitalin.....	1 mg. per kilogram
Sodium chloride solution.....	0.9 per cent, 10 cc. per kilogram

COURSE OF THE EXPERIMENTS

The average daily output of urine by each animal was determined at the end of the third day, during which time the preliminary observations were being made. Following the injection of uranium the daily output of urine was ascertained and compared with the average daily output by the animal prior to the use of uranium.

Three of the animals were used experimentally after they had developed a nephritis but before the development of a glycosuria. The daily output of urine by these nephritic and non-glycosuric animals showed a moderate increase as follows. The urine from the different animals increased respectively from 278 to 318 cc., from 392 to 440 cc. and from 386 to 358 cc. The urine showed qualitatively a pronounced reaction for albumen, and microscopically hyaline and granular casts and erythrocytes.

The remaining animals were used experimentally after the development of a glycosuria. In each instance, with the development of a glycosuria the output of urine at once enormously increased. For example, in experiment 1, in which the animal was receiving daily 350 cc. of water, the average daily output of urine for the three days prior to the uranium was 385 cc., while with the development of a nephritis and an accompanying glycosuria the urine increased on the first day to 620 cc. and on the second day to 750 cc.

Again, in experiment 8, in which the animal was receiving 500 cc. of water daily, the average output of urine prior to the uranium was 513 cc., while following the uranium and with the development of a nephritis and a glycosuria the output of urine increased to 1310 cc.

This increase in the output of urine was not an occasional occurrence, but it developed in each animal that was allowed a sufficient time to develop a glycosuria. These polyuric and nephritic animals were anesthetized by one of the methods previously mentioned. Within thirty-four minutes to an hour and a half after the commencement of the anesthetic, the output of urine from these excessively diuretic animals was either very greatly reduced, reduced to a condition bordering on an anuria, or an anuria had

developed which in six of the animals persisted throughout the experiment, uninfluenced by the diuretics which were employed.

This pronounced reduction in the output of urine after the anesthetic is equally as striking as is the increase in the output of urine after the animals have developed a glycosuria.

Experiment 20 is used to illustrate these observations. The animal was receiving 500 cc. of water daily. The average output of urine for the three days prior to the uranium was 464 cc. The animal was given subcutaneously one injection of uranium of 10 mgs. The animal rapidly developed a nephritis and a glycosuria, and the urine increased to 1018 cc. At the time of the experiment 294 cc. of this urine was found in the bladder, which shows quite clearly that the animal was diuretic until the time of anesthesia. The experiment lasted four hours and during this time the animal was in a perfectly satisfactory physiological condition. The general blood pressure varied between 93 and 108 mm. of mercury and the renal vessels were physiologically responsive to caffeine, theobromine and 0.9 per cent salt. Not a drop of urine was voided.

This experiment, associated as it is with others which give identically the same results, shows a definite relation between the polyuria and the development of glycosuria. Secondly, it shows an equally intimate connection between the use of an anesthetic and the development of an anuria. The polyuria in uranium nephritis and the influence of the anesthetic in reducing the output of urine has been observed by both Schlayer (2) and Pearce (3). Pearce attributes the anuria to a "decreased glomerular permeability" and makes a similar suggestion to interpret the results obtained by Schlayer. So far as I have been able to learn these authors make no note of the association of the polyuria with the onset of the glycosuria.

THE EFFECT OF DIURETICS IN URANIUM NEPHRITIS

To facilitate the study of the effect of the different diuretics the experiments have been classified into groups, e.g., the Anuric, Practically Anuric and Diuretic Groups.

Anuric group

Six experiments are included in this group. In all six of the animals caffeine, theobromine, and digitalin were employed as diuretics and in four of the animals 0.9 per cent salt was also used. None of these agents had any effect in reestablishing a flow of urine. This failure cannot be attributed to either a failure on the part of the diuretics to increase and maintain an adequately high general blood pressure for urine secretion, or to a failure in the vascular response of the kidney. The following experiment will serve well to illustrate these points:

Experiment 23. The animal's general blood pressure at the commencement of the experiment was 104 mm. of mercury and at the termination 107 mm. Caffeine produced a rise in arterial pressure of 4 mm. of mercury and a rise in the oncometer of 27 mm. (water manometer). Theobromine produced a rise of 7 mm. in general pressure, and a rise in oncometer pressure of 59 mm., digitalin a rise of 18 mm. in arterial pressure and 20 mm. in oncometer pressure, while 0.9 per cent salt caused no rise in general blood pressure, but a rise of 15 mm. in oncometer pressure. The animal remained anuric throughout the experiment.

Practically Anuric group

Falling in this group are experiments 6 and 11. They represent animals which are not absolutely anuric but which show a gradual decline in the flow of urine which is but slightly influenced by the diuretics.

The first animal of this series, experiment 6, prior to the anesthetic had an output of urine of 810 cc. Following the anesthetic an anuria developed for two hours, although during this time a rise of blood pressure of 14 mm. of mercury and of oncometer pressure of 12 mm. of water was obtained from caffeine and a rise of 10 mm. in general pressure and of 12 mm. in oncometer pressure from theobromine. During the last half hour of the experiment, under the effect of 0.9 per cent salt the arterial pressure rose 17 mm. and the oncometer pressure 20 mm. The urine filled the ureter canula and a few drops were discharged into the receiving flask.

Experiment 11 followed the same general course. Prior to the anesthetic the animal was highly polyuric. Following the anesthetic the output of urine for the first half hour period was 2 cc. The urine flow then decreased, although the animal showed the usual physiological response to caffeine and theobromine. During the final half hour period of the experiment the flow of urine had been reduced to two drops. The experiment demonstrates a continuance of those changes, whatever they may be, which lead to an anuria, and which commence with the administration of the anesthetic, and in this instance have progressed, uninfluenced by the employment of diuretics.

Diuretic group

In the animals classified as diuretic, the term is used relatively. With few exceptions these experiments were terminated artificially during a period of diuresis. Such a termination does not exclude the possibility of the animal later becoming anuric as was illustrated in the previously described experiment.

The following experiments are representative of this group:

Experiment 16. The animal had a pronounced nephritis, was polyuric and had developed a glycosuria. Following Gréhant's anesthetic the animal became anuric for forty-five minutes. Following the use of caffeine, with a rise of arterial pressure of 5 mm. of mercury and of oncometer pressure of 8 mm. the urine flow was reestablished and during the half hour period following the use of caffeine the flow of urine was 1.5 cc. Under theobromine without a rise in arterial pressure but with a rise in oncometer pressure of 4 mm., the flow of urine increased to 3 cc. in a half hour period. With digitalin which produced a rise in arterial pressure of 10 mm. and in oncometer pressure of 8 mm. the urine flow increased to 3.3 cc. in a half hour interval.

In three of the experiments of this series 0.9 per cent salt was used. With the salt solution the greatest degree of diuresis was produced and this diuretic effect from the salt was more constant than that from the other diuretics in this type of nephritis.

In experiment 19, the flow of urine in the half hour period prior to the use of salt solution was 0.9 cc. Following the salt with a rise in arterial pressure of 14 mm. and in oncometer pressure of 49 mm. the urine increased to 1.7 cc.

In experiment 17 with a flow of urine of 1.6 cc.—prior to the use of salt solution, following its use the urine increased to 4.6 cc. The oncometer pressure rose 18 mm. and the general pressure 6 mm.

The following deductions concerning the diuretic value of the different substances employed in these groups of experiments are as follows:

1. In the anuric group, caffeine, theobromine, digitalin and 0.9 per cent salt solution have no effect in reestablishing the flow of urine. Their failure does not depend upon their inability to raise and maintain a sufficiently high general blood pressure to produce diuresis.

2. The inactivity of these substances is not due to their inability to influence the local renal circulation, for the physiological vascular response of the renal vessels as indicated by the oncometer readings is normal or hyperactive.

3. In the group of experiments classified as practically anuric the same deductions concerning the inefficiency of the diuretics are allowable.

4. In addition to these deductions relative to the effect of the diuretics, this group also shows that the quantity of urine may not only not be increased by the diuretics, but that the output of urine may progressively decrease, even though the general blood pressure readings and oncometer readings show the usual response.

5. In the diuretic group in which the animals show the same physiological response to the diuretics as was shown by the animals in the anuric and practically anuric groups—the substances effect a diuretic action. Salt solution, 0.9 per cent, shows a more constant diuretic effect, and the increase in the flow of urine from the salt is more pronounced than it is from the other substances.

THE RENAL PATHOLOGY

Five of the animals used in this investigation were killed either prior to the anesthetic or during its administration. Four of these animals had an early uranium nephritis, were markedly

polyuric and had a glycosuria. The fifth animal had a late uranium nephritis, was glycosuric but was not polyuric. The output of urine was reduced below the normal.

In the four early nephritides the vascular pathology of the kidney was much more pronounced than was the epithelial pathology, while in the fifth animal with a late uranium nephritis in which the output of urine had been reduced below the normal, the epithelial pathology predominated. The vascular pathology in the early nephritides, consisted primarily in an acute engorgement of the glomerular capillaries. The hyperaemic capillary tufts usually filled the space enclosed by Bowman's membrane and frequently this structure gave the appearance of being distended by the enclosed capillaries. The endothelial nuclei of the capillaries and of the capsular membrane showed no degeneration but were unusually prominent. Within the capillaries the vacuoles first described by Christian (6) were observed in two of the kidneys.

The intertubular vessels showed the same engorgement with an occasional intertubular exudate containing a few erythrocytes.

With this pronounced vascular reaction on the part of the kidney the epithelial pathology was remarkably slight. The epithelium had not degenerated, it stained well and showed no encroachment upon the lumen of the tubules. (Figs. I and II.)

A comparison of the epithelial changes in these animals, with the epithelial changes in those animals having a complete anuria is as striking as is the difference in the output of urine by the two groups of animals before and after the administration of an anesthetic.

Four of the anuric animals were in an early stage of uranium nephritis, the stage which has just been described as existing in the animals killed before the administration of an anesthetic. In these animals with an early uranium nephritis which were polyuric and glycosuric, and which following the anesthetic became anuric, the vascular pathology was histologically similar to the vascular pathology noted in those animals that had not been subjected to the effect of an anesthetic. The epithelial pathology in these two groups of animals shows, however, a well

marked difference. The epithelium in the anuric animals is very greatly swollen and is usually vacuolated. As a result of the swelling the lumen of the tubules has either been very greatly encroached upon or the lumen has become obliterated by an apposition of the opposing faces of the tubular epithelium. The epithelial changes are most pronounced in the convoluted tubules. (Figs. III and IV).

In the animals grouped as practically anuric, the renal pathology is so nearly similar to the pathology of the kidney in the anuric group that the two allow no histological differentiation.

In the animals grouped as diuretic, the vascular pathology is similar histologically to the vascular pathology which has been described for those animals in the anuric group and also for those animals which were killed prior to the use of the anesthetic. The epithelial pathology, however, differs very much from the epithelial pathology of the anuric group but resembles in its appearance the epithelial reaction seen in those diuretic animals obtained before the use of an anesthetic. (Figs. V and VI.)

SUMMARY

1. Early in a uranium nephritis, usually within the first twenty-four hours, the animals develop a glycosuria and become markedly polyuric.

2. Following an anesthetic, morphine-ether, or Gréhant's, these animals either become completely anuric or the output of urine is greatly reduced.

3. Such animals under the effect of caffeine, theobromine, digitalin and 0.9 per cent salt solution, show a normal response in the general blood pressure rise and in the vascular response of the kidney.

4. In certain of these animals the flow of urine is increased by these diuretics, while in other animals the urine flow is uninfluenced.

5. Histologically the vascular pathology of the kidney is similar in those animals which show a diuretic effect and in those animals which remain anuric.

6. Those animals which remain anuric show a physiological vascular response on the part of the kidney vessels similar to the response which is obtained in the diuretic animals. The physiological and the pathological reaction of the kidney vessels in the anuric and in the diuretic animals are, therefore, similar.

7. The two groups of animals differ, however, in the degree of involvement of the epithelial element of the kidney. The anuric animals show an epithelial involvement which is severe and which results anatomically in an encroachment upon, or occlusion of, the lumen of the tubules, while in the diuretic animals the epithelial changes are less marked and are insufficient to produce a mechanical obstruction of the tubular lumen.

8. The pathology of the kidney of those animals with an early uranium nephritis which were examined prior to the use of an anesthetic showed a vascular pathology which in general was similar to the vascular pathology of the anuric, practically anuric and diuretic animals. The tubular epithelium of these animals, which were polyuric, showed but slight changes, and in their epithelial reaction the kidneys of these animals were more nearly comparable to the kidneys of the diuretic animals than they were to the kidneys of the anuric animals.

The physiological and anatomical observations which have been made in this investigation indicate that in a uranium nephritis the epithelial changes are more responsible for a reduction in the output of urine or an anuria than are the vascular changes. The way in which these changes influence the output of urine will furnish the basis for a subsequent investigation.

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FIGS. I AND II

The figures represent the kidneys of a nephritic, glycosuric and polyuric animal before the use of an anesthetic. The glomerular vessels fill and distend the surrounding capsule and show the presence of vacuoles in the capillary walls. The tubular epithelium shows occasional vacuolation, is but slightly swollen and has not encroached upon or occluded the lumen of the tubules. The tubules contain granular detritus. B. and L. obj. 3, oc. 1.

FIGS. III AND IV

The figures represent the kidneys of two animals which were excessively polyuric before the administration of an anesthetic. Following the anesthetic the animals become anuric. The anuria remained uninfluenced by the diuretics. The vascular pathology is histologically similar to the pathology described in the polyuric animals illustrated by Figs. I and II. The epithelial pathology, however, is strikingly different. The epithelium shows an acute swelling resulting in a nearly complete occlusion of the lumen of the tubules. The acute nature of the swelling of the epithelium is well shown in Fig. III. The anuria was uninfluenced by the diuretics. B. and L. obj. 3, oc. 1.

FIGS. V AND VI

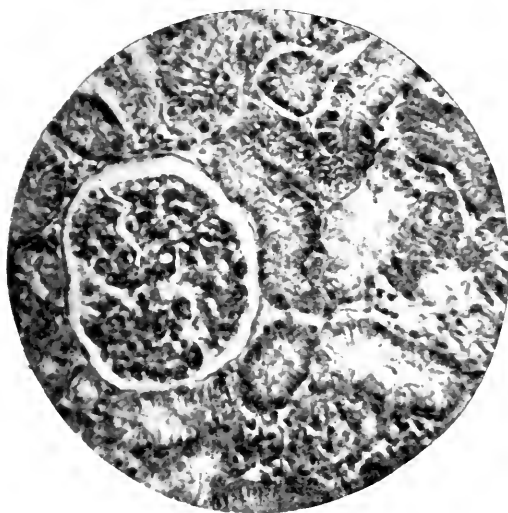
The figures represent the kidneys of two animals which were responsive to the diuretics. The vascular pathology is similar to that described in the anuric animals. The epithelium shows but slight swelling and no material encroachment upon the lumen of the tubules. B. and L. obj. 3, oc. 1.



I



II



III



IV



V



VI

ISOCALYCANTHINE AND ITS QUATERNARY BASE

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Calycanthine is an alkaloid found in the seeds of *Calycanthus glaucus* (Wildenow), a plant indigenous to the southern United States and known there as "Sweet Brush" or "Bubby." It is of economical importance because numerous cases of animal poisoning have occurred from cattle and sheep eating it. This alkaloid, according to Gordin, (1) has the formula, $C_{11}H_{14}N_2\frac{1}{2}H_2O$. It melts, crystalline at 216° to 18° C., anhydrous at 243° to 44° C., and loses its water of crystallization in a few hours at 120° C. without otherwise being changed by the heat. The pharmacology of this preparation has been studied by Cushny (2).

Having exhausted the material from which the calycanthine was prepared, Gordin ordered more seeds from the same dealer. These apparently were identical with those of the first sample and the alkaloid, obtained from them, appeared exactly like calycanthine. However, on analysis, this second alkaloid was found to melt, crystalline at 212° to 14° C., anhydrous at 235° to 36° C., and not to give up its water of crystallization by heating without partial decomposition of the base. That the crystalline form also contains water of crystallization is shown by its loss of weight when kept "in vacuo" over a drying agent such as sulphuric acid. Even such methods failed to bring the weight constant until after a period of twenty months, when the loss of water from a definite quantity of the crystalline substance agreed with the calculated formula, $C_{11}H_{14}N_2\frac{1}{2}H_2O$. Since this base has the same formula as calycanthine but differs in the points mentioned above, Gordin calls it isocalycanthine.

The alkaloid contains an NH group. The assumption was made, therefore, that it would react with methyl iodide to form compounds of the formulae, $C_{11}H_{13}(CH_3)N_2$ and $C_{11}H_{13}(CH_3)N_2CH_3I$, respectively. In no case was either of these bases found but, instead, a new and unexpected compound of the formula, $C_{24}H_{28}N_3IOH_2O$. The reaction is complicated and, from 10 grams of isocalycanthine, about 4 grams of the quaternary iodide is obtained. It is difficult to understand how a quaternary iodide, containing oxygen and three atoms of nitrogen in the molecule, can be obtained from an oxygen free base, having only two atoms of nitrogen. Gordin gives a probable explanation (3).

The new quaternary iodide is both a neutral ammonium salt and a weak monoacid base. It is colorless, difficultly soluble in water and combines with strong acids to form soluble salts of a light yellow color. With weak acids no such salts are produced, and from the yellow salt solutions, ammonia, fixed alkalies and ammonium carbonates reprecipitate the quaternary base.

The peculiar physical properties and chemical reactions of isocalycanthine made a pharmacological study advisable. Dr. Gordin kindly furnished us with a sufficient quantity of isocalycanthine hydrochloride, $C_{11}H_{14}N_2HCl \cdot H_2O$, and its quaternary chloride, $C_{24}H_{28}N_3ClO_3H_2O$, for this purpose. In carrying out this work we have followed more or less closely the work of Cushny (4) on calycanthine.

ISOCALYCANTHINE HYDROCHLORIDE

Action on the nervous system

The alkaloidal salt, in physiological salt solution, was injected into the abdominal lymph sac of 25 gram frogs. Cats were given the drug hypodermically.

Experiment 1. Frogs—5 mg. equal to 0.2 mg. per gram

November 9

- 2:10 p.m. Two frogs injected.
2:30 Sit erect, are lively and apparently normal.
2:35 Highly irritable.
2:40 Distinct clumsiness. Crawl instead of hopping but are still able to hop.
3:10 Bodily movement much impaired. Turn over when placed on back.

November 10

- 8:00 a.m. Less clumsy and highly irritable.
12:00 m. Still irritable. No indication of spasms.

November 11

- 8:00 a.m. Still irritable.

November 13

- 8:00 a.m. No. 1—Highly irritable. Appeared normal two days later.

November 24

- No. 2—Tetanic spasms with opisthotonus, continuing until death on November 24.

Experiment 2. Frogs—10 mg. equal to 0.4 mg. per gram

November 9

- 2:12 p.m. Two frogs injected.
2:25 Irritability increased.
2:40 Distinct impairment of motion. Crawl but have great difficulty in drawing up legs. Abduction of limbs similar to picrotoxin effect but less pronounced. Recover position with difficulty when placed on back.
2:45 Float in water. No curare-like action and no tremors discernible.
3:00 Marked loss of ability to draw hind legs up to the body.
3:10 Cannot turn over when placed on back.

November 10

- 8:00 a.m. Tetanic spasms.
3:00 p.m. Tetanus still persists. No. 3—Decapitated—spasms still continue but less marked. Death three hours later.
4:00 No. 4—Tetanic spasms, with extreme backward extension of the head. Died during the night.

Experiment 3. Frogs—20 mg. equal to 0.8 mg. per gram

November 9

- 2:15 p.m. Two frogs injected.
2:20 Bodily movements notably impaired. Respiration increased. Hind legs drawn up with great difficulty and usually remain abducted (as in picrotoxin effect).
2:27 Paralyzed. Perfectly limp. When placed on back, make no attempt to turn over. Leg reflex on stimulation.
2:45 Sink in water.
3:00 Hearts beating feebly.

November 10

- 8:00 a.m. No. 5—Dead.
No. 6—Weak strychnine-like spasms.
12:00 m. No. 6—Spasms only on stimulation.
4:00 p.m. No. 6—Spasms only on stimulation with extreme opisthotonus.

November 11

- 8:00 a.m. No. 6—Spasms only on stimulation with extreme opisthotonus.

November 13

- 8:00 a.m. No. 6—Continuous spasms with opisthotonus; increased on stimulation. This condition continued until

November 25

- a.m. Animal died.

Experiment 4. Frog—25 mg. equal to 1 mg. per gram

January 21

- 2:00 p.m. Injection.
2:05 Irritability increased.
2:07 Clumsiness, loss of ability to draw up legs.
2:15 Profound paralysis—no recovery. Died during night.

Experiment 5. Cat—Weight, 2.1 kilo. Dose. 4.8 mg. per kilo

November 15

- 1:35 p.m. Ten milligrams injected.
5:35 Nervous, easily angered. Clumsy, legs stiff and drawn up slowly. Walking difficult.

November 16

8:30 a.m. Irritable. Spasmodic jerks on tactile stimulation if sudden. Walks with abdomen close to the floor.

5:30 p.m. Normal. No symptoms appeared for a week.

Experiment 6. Cat—Weight, 3.1 kilo. Dose, 8 mg. per kilo

November 15

1:25 p.m. Twenty-five milligrams injected.

4:25 Slightly irritable, timid. Crouches in corner of cage. No spasms on stimulation.

5:25 On jarring the cage, animal has a single clonic spasm which apparently involves all muscles. Elicited repeatedly on stimulation and varies to a certain extent with the stimulus. The animal has some power of inhibiting these sudden contractions, especially when it sees the approach of the stimulus. Muscles between the spasms are in extreme tonus and tremors can be felt. Consciousness seems to be diminished. There are no eye or other symptoms that resemble morphine.

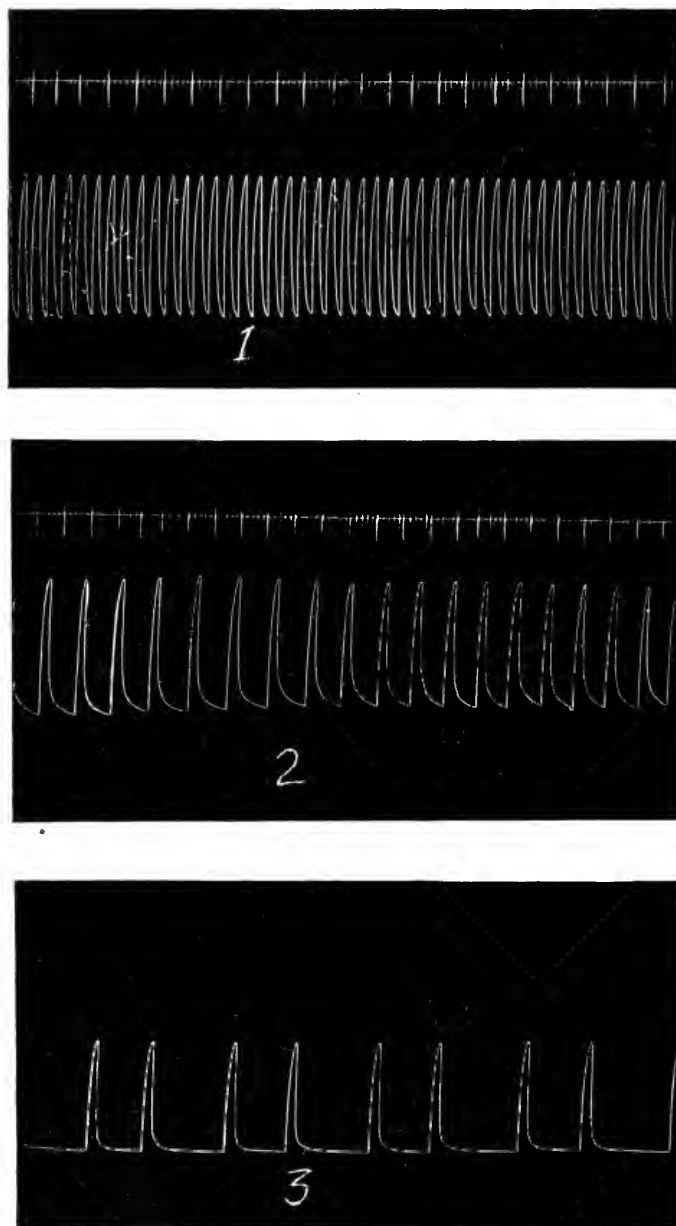
November 16

8:00 a.m. Animal found dead in cage.

Other frogs were pithed and injected with similar amounts of isocalycanthine, after ligation of the right leg under the exposed sciatic nerve, and the sciatics stimulated from time to time. The threshold stimulus of the left nerve was found to be somewhat increased and the corresponding limb became fatigued on tetanic stimulation much sooner than the ligated control.

The action in the frog, then, is increased irritability, followed by clumsiness and impairment of bodily movement. This is succeeded by a distinct paralysis, if the dose be large (over 0.2 mg. per gram frog); later convulsions appear, resembling strychnine spasms but differing in being long sustained and accompanied by extreme opisthotonus. Very strong doses may kill the animal without spasms appearing.

Cats show successively: increased reflexes, clumsiness and impairment of body movement with a peculiar stiffness of the legs



TRACING I. MYOCARDIOGRAM OF TURTLE'S HEART WITH 1 PER CENT ISOCALYCANTHINE HYDROCHLORIDE IRRIGATION

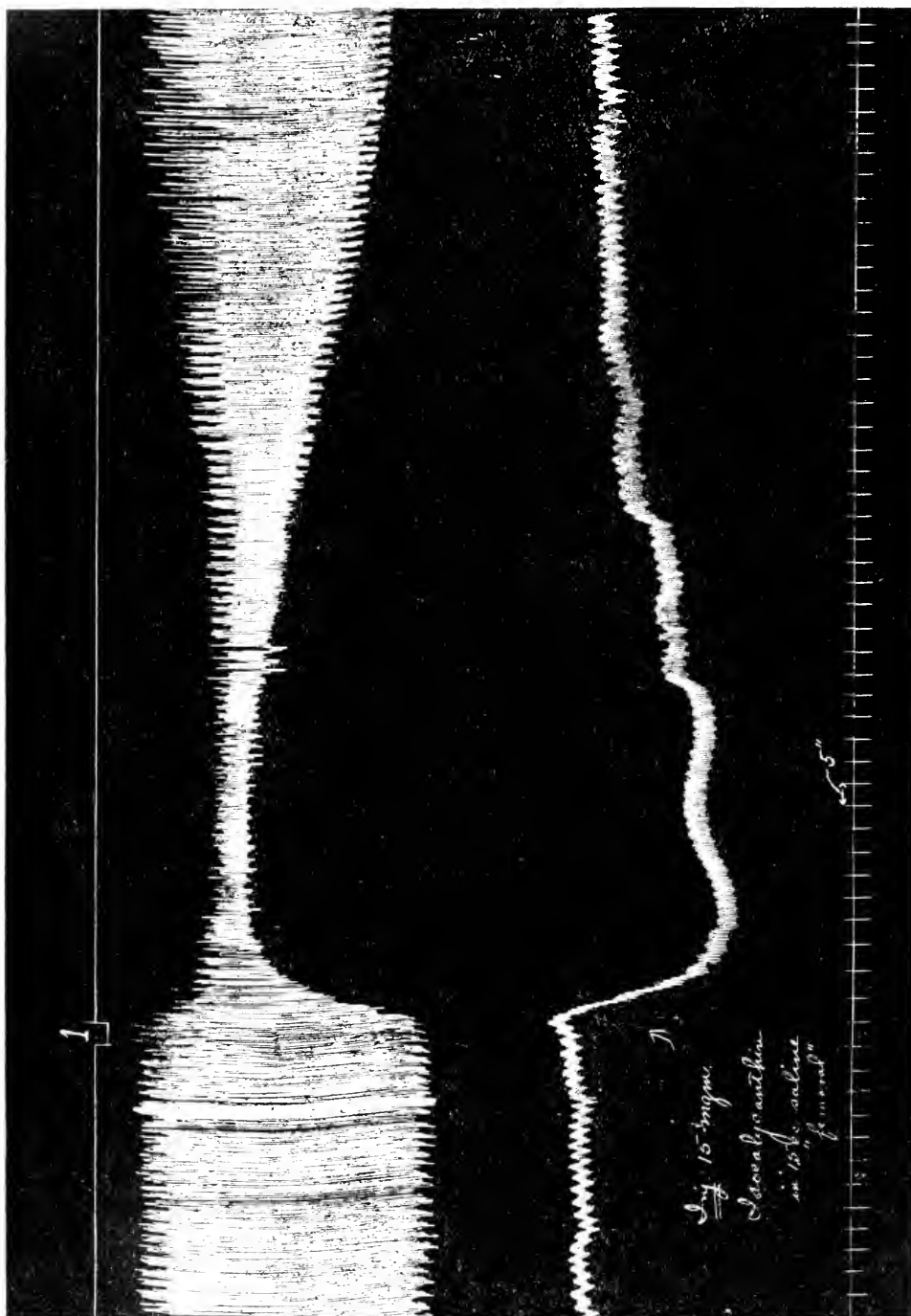
Suspension Method. 1, normal; 2, five minutes after irrigation with 5 mgm. of the drug; 3, after ten minutes irrigation.

in walking. Toxic doses further produce increased muscular tonus and extreme excitability so that the slightest stimulus causes the animal to execute a single spasmodic jump which, however, if anticipated, may be partially inhibited.

Action on the circulation

Heart tracings were made with the frog, turtle and cat. Blood pressure was taken from the rabbit, dog and cat. In general, the heart beat is greatly decreased, both in rate and amplitude, the effect being immediate but transient. Toxic doses stop the heart in diastole. Irrigation with even a few drops of 1 per cent isocalycanthine causes a perceptible slowing of the frog's heart, while larger doses stop it entirely. With turtles 5 mg. cause a pronounced slowing and 10 stop the heart quickly (tracing I). While the heart rate is rapidly altered in these two animals, it is to be noticed that the decrease in amplitude does not come on until later. In cats, however, both the rate and amplitude are simultaneously diminished directly after the injection. The heart becomes immensely dilated and the coronary vessels are distended with blood. If the dose is non-fatal, the immediate depressing effect lasts less than a half of a minute and is followed by a gradual recovery to normal within four or five minutes. A dose of 6 mg. per kilo stops the heart entirely. Repeated injections (ranging from 2 to 4 mg. per kilo) at five-minute intervals give uniform results except a slight decreased toxicity for each succeeding dose. The striking fact is that this toxicity is shown in the ventricles; the auricular beats, while visibly slowed, are always strong. Stimulation shows the vagi to be active and previous paralysis of the vagus endings by atropine, does not alter the isocalycanthine curve.

With blood pressure the effect varies with the dose. In one experiment with a 2-kilo rabbit 2 mg. caused a slight temporary fall; 5 mg. resulted fatally. In an 8-kilo dog, 200 mg. injected 20 mg. at a time in rapid succession, was accompanied by some fall in blood pressure which was temporary and did not prove fatal. There is produced in cats a marked drop in blood pressure which



TRACING II. BLOOD PRESSURE AND MYOCARDIOGRAM FROM CAT WITH ISOCALYCANTHINE HYDROCHLORIDE INJECTION
Ether, tracheotomy, carotid pressure, modified Roy-Adami cardiograph, injection in femoral vein. Weight 3 kilograms.
I, Injection of 15 mg.

tracing II shows to be exactly parallel with the depressing effect upon the heart. Tracing III demonstrates that the blood pressure may be maintained, during an injection, by epinephrine and that the heart depression persists independently of the arterial pressure. However, the fatal dose of isocalycanthine for cats is found to be considerably increased by giving epinephrine with the isocalycanthine.

Because of the shortness but intensity with which the alkaloid acts upon the circulation, attempts were made to determine the effect of injections other than by the usual method through the femoral vein. In introduction of the drug by way of the mesenteric veins or the femoral artery, the blood pressure curve is the same, except that the onset in the former comes on a few seconds earlier and in the second a half of a minute later. Isocalycanthine mixed with defibrinated blood drawn from the same animal gave similar results. It was found in addition that, after repeating a given dose several times, the effect of each successive dose becomes less and less so that the depression following the sixth or seventh injection may be only one-half or one-third of the original.

QUATERNARY CHLORIDE

This salt is difficultly soluble in water. To make a $\frac{1}{2}$ per cent solution of it, in physiological salt solution, a trace of HCl was added, the resulting reaction being a faint acidity to litmus. Control experiments show that this trace of acid may be disregarded.

Action on the nervous system

Frogs and dogs were used. These were injected in the same way as the animals in the series on isocalycanthine.

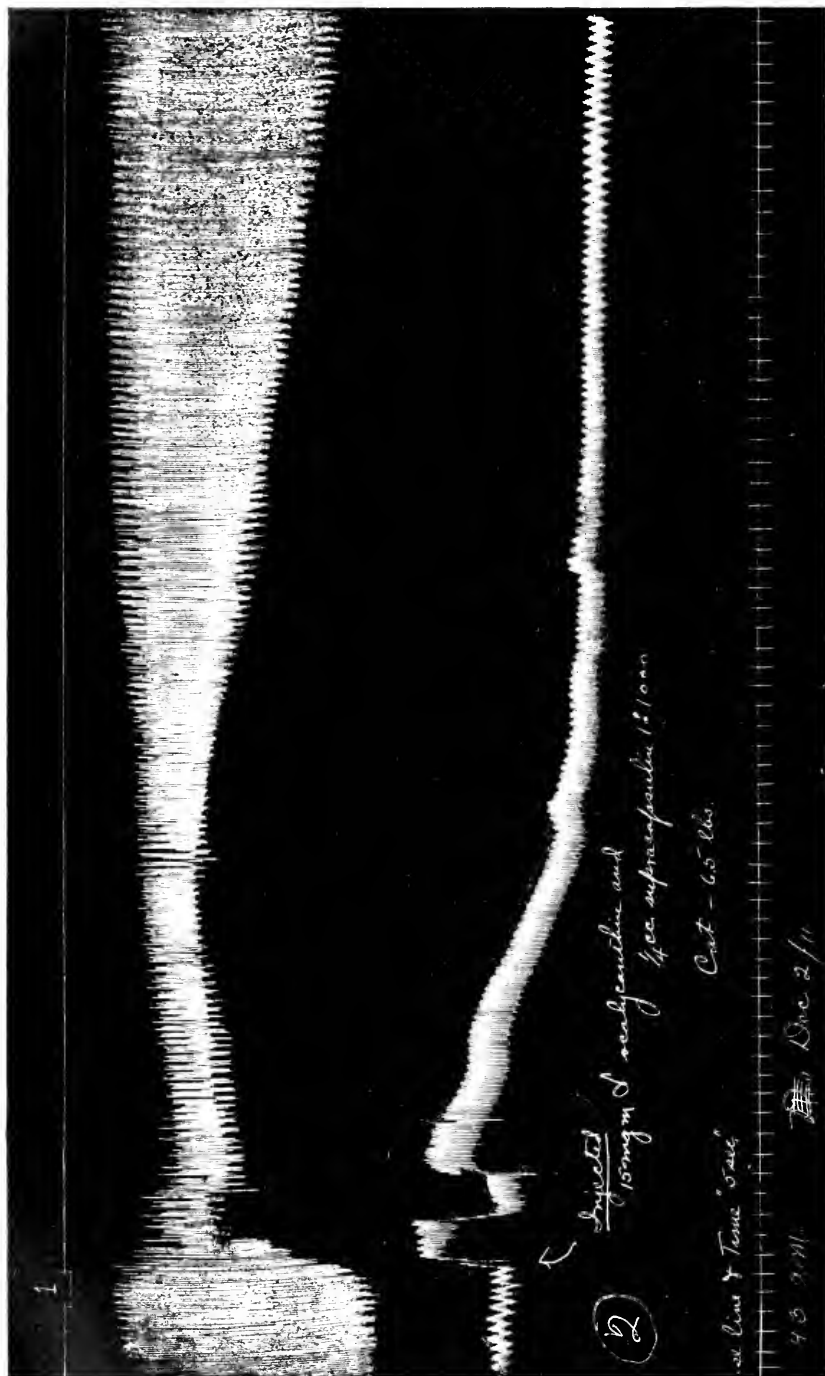
Experiment 7. Frogs—5 mg. equal to 0.2 mg. per gram

November 13

3:00 p.m. Two frogs injected.

3:10 Can hop but have great difficulty in drawing up hind limbs.

3:40 Move legs voluntarily when stimulated.



TRACING III. BLOOD PRESSURE AND MYOCARDIOGRAM FROM CAT WITH ISOCALYCANTHINE HYDROCHLORIDE AND EPINEPHRINE INJECTION
 Same animal as in Fig. II. *I*, Injection of 15 mg. of isocalycanthine and $\frac{1}{4}$ cc. epinephrine solution, 1:1000.

November 14

9:00 a.m. No. 1—Is able to move slightly.

No. 2—Appears lifeless but muscles respond to direct stimulation.

2:00 p.m. No. 1—Recovered. No. 2—Paralyzed.

November 16

8:00 a.m. No. 1—Recovered. No. 2—Dead.

Experiment 8. Frogs—10 mg. equal to 0.4 mg. per gram

November 14

3:04 p.m. Two frogs injected.

3:08 Distinctly paralyzed. Great difficulty in drawing up hind limbs. Muscles show a slight tremor.

3:14 Can move front legs but hind legs paralyzed.

3:20 Complete paralysis. Muscles contract on direct stimulation. Eyes dilated.

November 15

Paralysis.

November 16

8:00 a.m. Both dead.

Parallel experiments always gave the same typical paralysis. In one, both sciatic nerves were isolated for three-quarters of an inch, the left leg ligated under the exposed nerve to exclude the circulation, and the drug injected as above. After paralysis was pronounced, the normal limb responded to muscle stimulation only, while the ligated limb contracted on both sciatic and muscle stimulation. Isolated muscle-nerve preparations also gave similar results.

A young pup weighing 1050 grams was given 35 mg. of the quaternary base by mouth. In the following six hours and afterwards the animal was fairly lively, showed no awkwardness in movements and appeared entirely normal.

In frogs, then, injection is followed by impairment of motion, leading to a paralysis in which the somatic muscles respond to direct stimulation but not to nerve stimulation. When given per os to mammals no toxic signs develop.

Action on the circulation

The effect of the quaternary base on the heart of the frog, turtle or cat is negative even with large amounts. It is to be remembered in this connection that the drug is soluble only in fifty parts of water and so must be used in a relatively dilute form.

DISCUSSION

The isocalycanthine action is exerted mainly upon the central nervous system and the heart. Like many other drugs which stimulate the nervous system, it produces partial depression of peripheral motor nerve endings, when given in relatively large doses. The nervous effect is slow in onset and characterized by slight increased irritability, a pronounced subsequent depression, followed by convulsions which, however, may not appear for a considerable time. These resemble strychnine spasms in type but differ from the latter by the previous depression and late onset as well as the involvement of the higher nerve centers, the removal of which lessens but does not prevent the spasms. They are also similar to picrotoxin spasms in the way the legs are abducted but to a lesser degree. The long sustained contractions resemble those described by Harnack for the sulphides (5). The extreme opisthotonus especially involving the head, also points to an action upon the medullary centers. Apparently the higher brain centers are not involved until late since the frog retains its sense of position long after the onset of symptoms and the cat can voluntarily inhibit its exaggerated reflexes.

Isocalycanthine depression of the heart is immediate but transient. It is not affected by atropine and hence must be muscular. Only the ventricles are involved and the heart stops in diastole. The toxic effect lasts for a few minutes at most and repeated injections show that the action is not accumulative. Hence the drug may be (a) rapidly oxidized, (b) stored away in the other tissues, (c) be effective only while it enters the heart muscle (similar to Straub's theory (6) for epinephrine and muscarine action). The fall in blood pressure is simultaneous with the heart depression which occurs even when the blood pressure is maintained by epi-

nephrine, whence it follows that the lowering of pressure is not the cause but the effect of the heart action. Since normal defibrinated blood acting upon the alkaloid from ten to thirty minutes at 40° C. does not alter its subsequent action either in intensity or character, and, since injections, such that the drug passes either through the peripheral capillary or the portal circulation, alter the cardiac depression only in rapidity of onset, neither the first nor the second explanation accounts for the brevity of the action. Regarding the third explanation, it will be noted that, with repeated injections, a smaller depression is produced by each succeeding dose. But this is to be expected; if, as in epinephrine action, the toxicity of any given dose depends not upon the absolute concentration of the drug in the blood but upon the relative difference of concentrations between the blood and the "receptive substance."

The quaternary chloride has a typical curare-like action of paralyzing somatic motor nerve endings, but, like quaternary bases in general, has no definite action upon the heart or central nervous system.

CONCLUSIONS

1. Judged from the pharmacological action, calycanthine and isocalycanthine are identical.

2. The latter is a powerful but transient heart depressant, either when applied directly or when injected intravenously. The action is muscular since the effect persists with the maintenance of blood pressure and is not changed by atropine, or by cutting the vagi.

3. It causes a prompt temporary decrease of blood pressure which is the result of the simultaneous heart depression. The brevity of action is not the result of oxidation of the drug in the blood or its absorption into the other tissues but would seem to depend upon the factor of its penetration into the heart muscle itself.

4. In mammals the dose fatal to the action on the heart is increased by simultaneous injections of epinephrine.

5. The action on the central nervous system consists of a primary stimulation, followed, in frogs, by a marked secondary depression but leading again to an increased irritability which brings on spasms, resembling both strychnine and picrotoxin convulsions. In mammals these latter two stages are less evident, the second often being absent and the third stage showing exaggerated reflexes only. The lower brain centers as well as the cord seem to be involved, while the higher brain centers are not affected until late after the onset of symptoms.

6. A typical curare-like action is exerted by the quaternary base of isocalycanthine.

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SCIENTIFIC PROCEEDINGS OF THE AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

THIRD ANNUAL MEETING

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Edited by the Secretary, DR. JOHN AUER

The Toxicity of Caffein.^{1,2} William Salant and J. B. Reiger.
From the Pharmacological Laboratory, Department of Agriculture, Washington, D. C.

The toxicity of caffein in the rabbit varies with the mode of its administration, being least when given by mouth and greatest by intravenous administration. The toxicity is about from 15 to 20 per cent greater by subcutaneous injection than by mouth, but is about half of this when injected into the peritoneal cavity. No difference was observed in the toxicity of caffein whether administered into gluteal or into the lumbar muscles. When introduced by this route the toxicity was found to be less by one-third than when it is injected into the peritoneal cavity, but is about 30 per cent more toxic than the subcutaneous injection. White or black rabbits were found to be less resistant to caffein, than gray rabbits. The resistance of the guinea pig to caffein, as in the rabbit, is greatest when given by mouth. The minimum fatal dose is less by intraperitoneal injection but greater than by subcutaneous injection, thus differing from the rabbit in this regard. Cats are less resistant to caffein than guinea pigs or rabbits. The minimum lethal dose by mouth in the cat is the

¹ A detailed study will appear soon in Bulletin 148, Bureau of Chemistry.

² Presented by permission of the Secretary of Agriculture.

same as by subcutaneous injection, but is less when given by the intraperitoneal route. The toxicity of caffeine for white mice varies according to season, being twice as toxic in the early fall as in the spring, when resistance to caffeine is about the same as that of the guinea pig. But its resistance to caffeine in the fall was less than that of animals of other species employed in the present research. The tolerance of the white rat for caffeine is about the same as that of the pigeon. No effect of season was noticed. The toxicity was the same whether caffeine was introduced into the peritoneal cavity or into the subcutaneous tissues. The minimum fatal doses for dogs was found to be the same by mouth as by subcutaneous injection, and is almost the same as for the cat. The resistance of the pigeon to caffeine is very much the same as that of the herbivora, but it approaches more nearly the guinea pig than the rabbit in this regard. The toxicity varies much more in different individuals when given by mouth than when injected subcutaneously. The toxicity of caffeine varies in the guinea pig according to season and this seems to be the case also in mice. Age is likewise a factor in the toxicity of caffeine, young animals being more resistant than the full grown and older animals. This was shown in experiments on rabbits, cats and dogs. The symptoms of caffeine poisoning were also different in puppies and full grown dogs. Diet such as carrots and oats did not influence the resistance of rabbits and guinea pigs to caffeine. Low protein diet tends to decrease resistance to caffeine in dogs. Young growing dogs are less resistant to caffeine on a meat than on a milk diet. Caffeine is not cumulative in the rabbit or dog, even if administered for a considerable length of time. A mild tolerance may be induced in the rabbit under certain conditions, but not in dogs under the condition of the experiments made in this investigation. The possibility, however, that dogs may acquire tolerance for caffeine is not excluded. Although the rabbit tolerates a much larger single dose of caffeine than the dog, it was found, in experiments on chronic caffeine intoxication, that the rabbit is less resistant to caffeine than the dog. The toxicity of caffeine is increased under some pathological conditions, since comparatively smaller doses proved

to be fatal to rabbits, cats and dogs, when marked lesions not due to caffeine were found at autopsy. Glycosuria was observed in rabbits, guinea pigs and cats when caffeine was given in sufficient amounts.

The Physiological Action of Salts of Choline. By R. R. Renshaw, Frank P. Underhill, and Lafayette B. Mendel. From the Chemical Laboratory of Wesleyan University, Middletown, Conn., and the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.

The salts of choline and related compounds have lately attracted attention in view of their observed occurrence in animal tissues and possible significance as hormones. Choline salts have generally been stated to exhibit a transitory depressor effect on the circulation; but Popielski and Modrakowski have ascribed this to the action of contaminating substances or decomposition products and insist that *pure* choline salts exert a pressor effect, if anything. Renshaw's investigations on the synthesis of choline have given us opportunity to make a physiological study of exceptionally pure synthetic choline salts with the outcome that *we have never failed to observe the characteristic transitory fall of arterial pressure.* This is not profound or prolonged, but is never absent even when fractions of a milligram of choline salts are injected. The "contamination" theory is rendered improbable by the fact that choline salts showed no quantitative differences in this physiological effect when different specimens of presumably unequal purity, separated by fractional precipitation, were investigated. One milligram of odorless choline chloride obtained after fifteen fractional precipitations gave the usual typical response.¹ Products purified by the platinic chloride separation especially recommended by Modrakowski showed no diminution in their depressor characteristics. If, as Popielski claims, a deteriorating decomposition readily sets in, this is contrary to our experience; for pure odorless salts preserved nearly two years showed no quantitative exaggeration of their capacity

¹ Cf. Mendel and Underhill: *Zentralblatt für Physiologie*, 1910, xxiv, No. 7.

to lower blood pressure. The fall of pressure can therefore scarcely be attributed to decomposition products, since it is doubtful if properly prepared and preserved choline salts readily decompose.

The investigation has included a study of both the chloride and sulphate of choline. The typical depressor reaction is abolished by atropin, but not by cutting the vagi. Our experience in general agrees with the recently published observations of Lohmann, and Abderhalden and Müller in indicating that the views promulgated by Popielski and his pupils about choline salts are not tenable.

The Seasonal and Sex Variations of Rana pipiens to Digitalis.

Worth Hale. From the Pharmacological Laboratory, United States Public Health and Marine Hospital Service.

A question of importance in connection with the use of frogs as test animals in assaying the digitalis series of heart tonics is that of their variability dependent on sex and season. In the course of the routine examination of digitalis during a period of three years, observations have been made on both these points which are not in accord with the reports made by certain European investigators, Schmiedeberg, Focke, Dixon and others, to the effect that in both these particulars the European frog *Rana temporaria* varies from 30 to 100 per cent in its reaction.

In my work *Rana pipiens* was used exclusively, the animals being received at different seasons from the dealer and were kept at the laboratory in wet cages at a temperature of 10° to 15° Centigrade. They were brought from the tanks to the operating room and tested at a uniform temperature of 22° Centigrade in their reaction to various digitalis preparations by the one hour method. The results of tests made at different seasons as shown by the following table indicates that *Rana pipiens* does not vary in reaction over 10 per cent, the limit of accuracy of the method, in activity from March to December, inclusive.

Females even at the mating season have not, on account of their greater weight from the enlargement of the egg sac, been

found more susceptible to digitalis when the dose is estimated per gram of body weight.

PREPARATION	MARCH TO APRIL	JULY TO AUGUST	NOVEMBER TO DECEMBER
Digitalin (French).....	0.000,013	0.000,012	0.000,012
Digitoxin.....	0.000,008,5	0.000,008	0.000,008,5
Strophanthin.....	0.000,000,55	0.000,000,525	0.000,000,5
Digitalin (German)	0.000,022	0.000,021	0.000,023
Tincture Digitalis I.....	0.007,5	0.007,5	

The Action of Diuretics in Uranium Nephritis. Wm. deB. MacNider. From the Pharmacological Laboratory, University of North Carolina.

The following summary is based upon results obtained in nineteen animals (dogs) with a uranium nephritis. Four animals were used as controls.

Early in a uranium nephritis there is a slight increase in the total output of urine. At this stage of the nephritis the epithelium is but little involved.

Usually within the first twenty-four hours of the nephritis the animals develop a glycosuria. The nephritic animals then become highly polyuric.

Following an anesthetic, either morphin, ether or Gréhant's mixture, the animals either become completely anuric or the output of urine is greatly reduced.

Such animals under the influence of caffein, theobromin, digitalin and 0.9 per cent salt solution, show a normal response in the general rise of blood pressure and in the vascular response of the kidney.

In certain of these animals the flow of urine is influenced by these diuretics, in others it is uninfluenced. The most marked and most constant diuretic effect is obtained from 0.9 per cent sodium chloride.

In general, the vascular pathology is similar in the diuretic and anuric animals.

In the anuric animals the epithelium has either greatly encroached upon or occluded the lumen of the tubules, while in the diuretic animals the encroachment of the epithelium upon the tubular lumen is less marked.

In the four nephritic and polyuric animals which were killed prior to an anesthetic the vascular pathology is in general similar to the vascular pathology seen in the anuric and diuretic animals. The renal epithelium, however, not only shows no swelling, but provided the kidney be from an animal with an early uranum nephritis the epithelium shows a relative shrinkage. For details see paper by the author in this number of the Journal.

Intramuscular versus Subcutaneous Absorption. L. G. Rowntree and J. T. Geraghty. From the Pharmacological Laboratory, The Johns Hopkins University.

To appear in an early number of the Archives of Internal Medicine.

Peritoneal and Pleural Absorption. W. E. Dandy and L. G. Rowntree. From the Pharmacological Laboratory, The Johns Hopkins University.

By determining the quantitative excretion of phenolsulphone-phthalein in the urine, it was shown that the absorption from the upper part of the pleural and peritoneal cavities is much greater than that from the lower part of these cavities.

Studies of Renal Function in Experimental Nephritides. L. G. Rowntree and R. Fitz. From the Pharmacological Laboratory, The Johns Hopkins University.

By means of various functional tests it is possible to determine in experimental nephritides whether the vascular or the tubular system is involved. In cases where both systems are involved, it is possible to demonstrate which one is preponderantly diseased. The tests are now being applied in the clinic in an effort to classify the various forms of nephritis.

Two Crystalline Pharmacological Agents Obtained from the Tropical Toad, Bufo aqua. John J. Abel and D. I. Macht.

Has appeared in no. 3, vol. iii of this Journal, pp. 319-377.

Upon the Action of Protein Poison. C. W. Edmunds. From the Pharmacological Laboratory, University of Michigan.

The work was carried on with the toxic portion of the protein molecule prepared according to the method of V. C. Vaughan, by treating the protein (in this instance casein) with an alcoholic solution of NaOH. Injected into frogs the general symptoms were those of depression. There was an almost complete paralysis of the motor nerves, similar to that produced by curara. The exposed heart showed very little change in rate, but was considerably weakened. Most of the work upon mammals which has been carried out up to the present time has been done on dogs. These show a very considerable fall in blood pressure when doses of about 100 mg. are injected. The pressure may fall as low as 25 mm. Hg, and this low pressure is associated with a paralysis of the vasomotor nerves in the splanchnic area. The point of action would seem to be upon the nerve terminations rather than upon receptive substance or muscle. Adrenalin will raise the blood pressure, as will also digitalis and barium chloride. Digitalis will raise the pressure to a higher point than it was before the casein poison was given, but whether it does so by constricting the paralyzed vessels has not been fully ascertained as yet; but it is believed that other factors, such as the action upon the heart, aid in the recovery. Investigations are being carried on at present as to the effect of the casein product upon the heart, and the rôle played by this organ in producing the fall of blood pressure.

There is very little effect upon the respiration, merely a slight acceleration, probably secondary to the circulatory disturbance.

The action upon the cat as far as examined, resembles very closely that upon the dog. The symptoms produced in the dog are quite similar to those described by Biedl and Kraus, as being characteristic of anaphylactic shock in that animal.

Chronic Chloral Poisoning. George B. Wallace. From the Pharmacological Laboratory, The University and Bellevue Hospital Medical College.

Experiments were described in which gradually increasing doses of chloral were given daily by the stomach to dogs. As chloral is changed in the body to trichlorethylalcohol, and this unites with glycuronic acid, forming the inert urochloralic acid, it was thought that the formation of this combination would keep pace with the increasing amount of chloral given, and by means of this protective agency a tolerance be established. The results of the experiments may be summarized as follows:

1. The earlier doses of chloral produce vomiting, but later the stomach becomes tolerant to the drug and vomiting does not occur. This local tolerance is common to gastric irritants. When large doses, 5 grams or more, are given daily, a gradually increasing looseness of the bowels results, which eventuates in a diarrhoea. The chloral given, however, is absorbed.

2. As far as symptoms of depression of the nervous system are concerned only a slight degree of tolerance is obtained. After doses sufficient to induce narcosis are reached, the gradual increase in dosage still brings about complete narcosis, but its duration becomes less. The tolerance of the nervous system is not marked therefore and is comparable to that from alcohol.

3. As the dose is increased no change in metabolism, as measured by total nitrogen and urea is seen, and the animal maintains a fairly constant weight. After daily doses of 5 grams or more, the ammonia rises however, and may reach twice the normal figure. The urine gives no qualitative reactions for acetone, or diacetic or oxybutyric acid. Albuminuria finally appears. The urine contains no sugar.

4. The chloral excretion takes place through the kidney and was calculated quantitatively by the amount of organic chloride in the urine. The exact forms in which it is excreted, i.e., the relative amount of free chloral, trichlorethyl alcohol, etc., have not yet been determined. Calculated as chloral from the organic chlorides eliminated, with small doses from 70 to 85 per cent of

the chloral ingested is excreted within eighteen hours. As the dose increases this percentage increases slightly. A breaking up of the chloral molecule, therefore does not occur.

5. The glycuronic acid excretion as determined by the method of Tollens runs fairly parallel to that of the chloral. Thus after a dose of 2 grams chloral, 1.73 grams chloral and 1.35 grams glycuronic acid were excreted. After 8 grams chloral, 7.21 grams chloral and 6.21 grams glycuronic acid were excreted. With the smaller dose therefore, 0.75 grams chloral and with the larger 2.6 grams chloral were not paired with glycuronic acid.

It may be said in conclusion that although the change of chloral to trichlorethylalcohol and the pairing of this with glycuronic acid is of the nature of a protective mechanism, it can not in itself bring about any marked tolerance for the drug, and rather is analogous to the power of the body cells to destroy morphin in cases of morphine tolerance.

On the Duration of Irritability of the Motor Nerves of the Frog after Death and the Effect of Strychnin upon It. T. S. Githens and S. J. Meltzer. From the Department of Physiology and Pharmacology, Rockefeller Institute for Medical Research.

The statements in the physiological literature regarding the duration of irritability of the motor nerves of frogs, refer to nerve muscle preparations kept in frogs saline or in Ringer solution. That method however, can not be used for a study of the after action of strychnin upon surviving frogs nerves, since the solutions might wash out the alkaloid. We studied the surviving irritability of the sciatic nerve in legs which retained their skins and in most cases remained in their natural connection with the body of the frog. The normal control frogs were killed either by destruction of brain and cord or by excision of the heart. In the strychnin frogs the heart was either intact or was excised before the injection. In a number of experiments the sciatic nerves of amputated legs were studied. The dead frogs were kept moist in the refrigerator and were taken to the laboratory only once or twice a day for the purpose of testing the irritability.

The frogs belonged to the species, *Rana pipiens*. The experiments were carried out in the last few months.

The following are our chief results. The sciatic nerves of animals which received no strychnin, remained irritable for seven or eight days; the muscles remained irritable one to three days longer. In frogs which received strychnin, the duration of the irritability of the sciatic, is in a general way, in inverse proportion to the dose; thus in frogs which received about 2 mgs. of strychnin per gram frog, the irritability was generally lost in less than twenty-four hours, and in many instances the peripheral irritability was practically nil a few hours after the reflex response was lost. This is true whether the heart was intact or removed. Since the loss of irritability of the nerve, probably means in this case that loss of irritability of the nerve ending, it is evident that in the latter case, the strychnin spreads from the place of injection to the periphery by the peripheral mechanism for distribution, that is through the lymph spaces. The irritability was lost early even if the sciatic nerve was cut previous to the injection, which shows that the migration does not necessarily take place through the lymph spaces of the nerve trunk. Except in two or three instances, it has never happened that the irritability was markedly reduced while the animals still showed any sign of life, although 2 mg. of strychnin per gram frog, is surely a large dose. In other words, while strychnin is capable of profoundly depressing the irritability of the motor nerves of the frog, which normally lasts long after death, we have not seen that, in the species of frog we have experimented with, it exerts a curare like action upon the motor nerves, while the animals are still alive.

The Effect of the Digitalis Group Including Anaphylaxis upon Some Skeletal Muscles in Rabbits, Especially with Regard to Rigor. J. Auer. From the Department of Physiology and Pharmacology, Rockefeller Institute for Medical Research.

In another communication¹ it was shown that the acute lethal cardiac changes in anaphylaxis of the rabbit closely resemble

¹ Proceedings of the American Physiological Society, December, 1911, Baltimore meeting. Amer. J. of Physiol., 1912, xxix, No. iv, p. xvi.

those which are produced by lethal doses of the ordinary members of the digitalis group, and for this reason, the changes which serum causes in the skeletal muscle of sensitized rabbits are included in this report.

Lethal doses of digitalis preparations when given subcutaneously, intraperitoneally, or intravenously cause a marked loss of irritability of the skeletal muscles, so that usually within fifteen minutes indirect and direct stimulation causes no response. Rigor, however, is not established until about one hour has elapsed.

In rabbits dead from acute anaphylaxis a fair irritability of the thigh muscles may persist up to one hour. Rigor, however, is usually well established within ten minutes in the arms, and in the legs within three-quarters of an hour.

In controls killed either by a blow on the neck, by medullary puncture or by bleeding, the thigh muscles remain irritable longer than one hour, and rigor is not well developed until after about two hours.

It is thus shown that digitalis and especially anaphylaxis definitely hasten the onset of rigor.

On a Difference between the Effects of Intravenous and Intra-aortic Injections of Curarin in Frogs. D. R. Joseph and S. J. Meltzer.
From the Department of Physiology and Pharmacology, Rockefeller Institute for Medical Research.

The most generally known action of curare is the paralysis of the motor nerve endings. This knowledge is derived from intravenous injections into mammals and injections into the lymph sacs of frogs. The older literature contains also many contradictory statements regarding the action of curare upon the central nervous system. When one leg of a frog was excluded from the peripheral action of curare by the Claude Bernard method, tetanic convulsions of that leg were occasionally observed and also late paralysis of reflexes was noted. About twenty years ago Tillie studied the entire subject with the pure alkaloid curarin prepared by Boehm. Among other experiments, Tillie injected curarin into

one of the aortae after tying the other aorta and also the aorta abdominalis communis. Convulsions appeared very soon and lasted for some time. Tillie thinks the convulsions were due to the fact that by this method a larger dose of curarin was brought in contact with the cord. He looks upon this method simply as an intravascular application. Tillie does not mention the fate of the motor nerve endings in these experiments of which only one protocol is given and it is not stated how many others were made. We are not aware that Tillie's experiments were ever repeated. On the other hand, the numerous intravenous injections of curare into mammals for the purpose of paralyzing the skeletal muscles never led to convulsive movements which were not due to asphyxia.

Our experiments were made on frogs, mostly *Rana pipiens*, with curarin (Schuchardt). The chief experiments consisted of, (1) injections into the common aorta without any further ligation and (2) injections into the abdominal vein. While both are intravascular injections and in both methods practically the same amount of curarin may be carried to the cord as well as to all other parts of the body, the modes of injection differ in one point, namely, that after the intra-aortic injection, the circulation ceases, while it continues to be active after the intravenous injection. We shall mention here only the chief results. In fifteen frogs which received intra-aortic injections of curarin, convulsions set in between a fraction of a minute and six minutes after the injection, lasted between one and seven minutes and were in most cases of a fairly strong character. The larger the dose the sooner the convulsions seemed to set in, but the shorter their duration and the milder their character. The cord became paralyzed, never to recover, between thirteen to twenty-two minutes after the injection. The paralysis of the motor nerve endings, however, appeared quite late. The earliest was sixty-three minutes while the longest was about twenty-four hours. In fourteen frogs, in which the injections were given into the abdominal vein, we see another picture. In six there were no convulsions at all and in the remaining eight they generally occurred late, in one case as late as one hundred and eight minutes after the injection; they also lasted longer than in the intra-aortic animals and were

generally of moderate intensity. The reflex irritability of the cord persisted fairly long, forty minutes being the shortest and one hundred and eighty-five the longest period of its duration. On the other hand the paralysis of the motor nerve endings made its appearance in every case quite early; fifteen minutes being the longest and two minutes the shortest interval between the injection and the appearance of the peripheral paralysis. In other words the intra-aortic injections favor an early appearance of convulsions of short duration, followed by a comparatively early paralysis of the cord while the peripheral paralysis sets in very late. The intravenous injection, on the other hand, favors a very early onset of the peripheral paralysis, a retarded development and a somewhat protracted period of convulsions, and a fairly late appearance of the paralysis of the cord. The experiments were varied in many ways but we shall not enter upon details. Neither shall we try at this place to offer a theory for this difference. We may only state that the hypothesis, that the continuous circulation may contain a mechanical or a chemical principle, or both, which may act as an elective factor, was the starting point of this investigation.

Comparison of Anesthesia in Plants possessing a Motor Mechanism and in Animals. G. W. Crile and M. L. Menten. From the Department of Surgery, Western Reserve Medical College.

In peripheral nerves after exposure for varying periods of time to vapors of the various fat solvent anaesthetics, e. g., chloroform, ether and ethyl alcohol, there is an increase in the amount of potassium in the medullary sheaths as shown microchemically by the potassium reagent of Macallum. A similar increased amount can be demonstrated as the result of mechanical or chemical injury. In those plants possessing a motor mechanism, e.g., *Mimosa pudica* and *Dionaea muscipula*, after exposure to the same fat solvent anaesthetics there is a marked increase in the demonstrable potassium compounds. This increase occurs in the guard cells, in the chlorophyll granules, in certain modified conducting elements, but to the greatest extent in those areas of the plant which are most active in producing motion and which,

upon stimulation show a considerable turgor. Lipoid substances as demonstrated by osmic acid and scarlet red have the same distribution as the potassium compounds. In plants as in animals the lipoid substances which contain potassium, e. g., lecithin and cholesterin after the application of these anaesthetics become so altered in their physical constitution that the contained potassium compounds can enter into the chemical combination with the reagent applied.

The Electrocardiogram in Morphin Poisoning in the Dog. J. A. E. Eyster and W. J. Meek. From the Department of Physiology, University of Wisconsin.

Intravenous or subcutaneous injections of toxic doses (30 to 90 mgs.) of morphin, causes as a rule a transitory increase in pulse rate followed by a slowing. During the period of slow pulse the rhythm may be regular or irregular. As a rule, pronounced irregularities appear first during partial recovery from the drug. The electrocardiographic records indicate that the slow regular or irregular pulse is due to a condition of sino-auricular and auriculo-ventricular heart block, separate or combined. This action of morphin is rapidly and completely antagonized by atropin and during the stages of recovery under the influence of this drug, the heart passes through various stages of sino-auricular and auriculo-ventricular block.

*The Effect of Caffein on the Circulation.*¹ William Salant. From the Pharmacological Laboratory, Department of Agriculture, Washington, D. C.

The intravenous injection of 15 to 25 mgs. caffein per kilo in animals was followed by a fall of blood pressure amounting to 7 to 35 per cent in most cases, which was however transitory in character. In some animals the blood pressure remained unchanged after such doses. A moderate rise of blood pressure was rarely observed. Experiments were also conducted to test the effect of various anesthetics on changes in blood pressure caused

¹ Presented by permission of the Secretary of Agriculture.

by caffeine. Some experiments were also made under local anesthesia.

Small doses of caffeine aid the action of nitrites on the circulation. A secondary rise was observed in a small number of cases under these conditions.

The effect of acetanilide is markedly increased when caffeine is given at the same time or when injected soon after. Increased rate and irregularity of heart action was also observed when acetanilide was followed by caffeine. Similar results were observed when caffeine was injected intravenously after ethyl or amyl alcohol but depression of the circulation was more marked after the latter. BaCl_2 injected intravenously after previous administration of caffeine was found to be toxic even in very small quantities and the blood pressure dropped suddenly. 0.5 to 1.0 mg. per kilo proved fatal, while much larger quantities of this salt administered to controls caused some disturbance of the circulation, but was not fatal.

*Demethylation of Caffeine and Theobromin under Pathological Conditions.*¹ William Salant and I. K. Phelps. From the Pharmacological Laboratory, Department of Agriculture, Washington, D. C.

Experiments on rabbits show that demethylation of caffeine and theobromin is retarded in chronic alcoholism. The inhibitory effect on demethylation persists at least several days after alcohol is withdrawn. Demethylation seems to be increased in pregnancy as shown by one experiment in which the amount of purin was enormously increased in this condition after the administration of theobromin.

*The Elimination of Caffeine.*² W. Salant and J. B. Reiger. From the Pharmacological Laboratory, Department of Agriculture, Washington, D. C.

The elimination of caffeine was studied in rabbits, guinea pigs, cats and dogs. Herbivora, especially rabbits, may eliminate as

¹ Presented by permission of the Secretary of Agriculture.

² Presented by permission of the Secretary of Agriculture.

much as 14 per cent of the amount administered, but, in most cases, 6 to 8 per cent of the caffeine given were recovered in the urine. Caffeine was also found in the stomach, intestines and bile. Rabbits and guinea pigs, which were fed carrots, eliminated larger amounts of caffeine in the urine than those which were fed oats. When the latter is eaten, much larger quantities are on the contrary found in the gastro-intestinal canal than in the urine. Elimination of caffeine in the urine begins fifteen to forty minutes after its subcutaneous injection and continues forty-eight to seventy-two hours, but the amounts found at this time are small. In some animals none could be found after twenty-four hours. Carnivora eliminate very small quantities of caffeine—about 1 per cent only.

The Action of Quinine on Leucocytes. George B. Roth. From the Pharmacological Laboratory, University of Michigan.

The administration of quinine, either as the sulphate or hydrochloride, to man, the dog, or the cat, was found to produce changes in the number of leucocytes which, when animals were used, could be divided into three distinct phases.

The preliminary leucocytosis, or first phase, appeared about an hour after the drug was given, and was slight. At this time the increase was mainly a lymphocytosis and was thought to be due to mechanical factors, namely the contraction of the spleen and lymphatic tissues.

In about an hour the second phase occurred, showing a reduction in the number of white cells, and affecting the lymphocytes mainly. This stage lasted several hours, and was followed by the third stage, which showed a rather marked leucocytosis. At this time the polymorphonuclear cells were increased, while the lymphocyte count remained low. When man was the subject of the experiment the three stages were not so distinct, but the differential count showed changes similar to those found in the cat and the dog.

On the Convulsant Action of some Dyes. David I. Macht. From the Pharmacological Laboratory, the Johns Hopkins University.

Barbour and Abel (this Journal, vol. ii, no. 3, December, 1910, p. 167) have called attention to a striking action of acid fuchsin on frogs. This substance injected into frogs in doses of 1 to 4 mgs. per gram of body weight, produces flexor spasms and convulsions, appearing in from one to twenty hours after injection, which in their turn give way to an extensor tetanus. Furthermore, and that is the most interesting part of their observations, these convulsions and tetanus can be produced much more quickly (in from one-half to thirteen minutes after injection) and by much smaller quantities of the dye (as little as 0.35 mg. per gram of body weight), if the anterior third of the cerebrum be cut off from the rest of the nervous system either before or after the injection. To explain this phenomenon these observers assume an inhibitory influence to pass from the cerebral lobes to subcortical coördinating centers, which inhibitory influence is cut off by the removal or division of the anterior third of the brain.

In the present research experiments were undertaken to investigate whether the above pharmacological properties are peculiar to acid fuchsin alone, or are exhibited by other substances.

The writer has studied with this end in view a large number of drugs (the various phenols, pikrotoxin, strychnine, atropine, etc.), and dyes (carmine, methylene blue, methyl orange, methyl violet, varieties of Congo red, different kinds of scarlet, red, tropaeolin, phenolsulphonephthalein, naphthol yellow, etc.) and has thus far found an exactly analogous action to that of acid fuchsin exhibited by three other substances—phenolsulphonephthalein, naphthol-yellow, and tropaeolin 00. These three dyes will produce convulsions and tetanus in frogs after given doses and in a given time, but the convulsions and tetanus will be produced by much smaller doses and in a much shorter time after injection if the anterior third of the brain is cut off or divided before or after the injection.

Large quantities of phenolsulphonephthalein (one or more milligrams per gram weight) were found to produce either no effect

at all or weak convulsions one hour or more after injection. On dividing the anterior third of the cerebrum as little as 0.5 mg. per gram weight produced violent convulsions and tetanus in from thirty seconds to thirty-five minutes after injection.

Injections of 2 mgs. or more per gram of body weight of naphthol yellow had no effect, or produced convulsions one or more hours later. Violent convulsions and tetanus were produced in decerebrate frogs in from one to twenty-eight minutes by doses as small as 0.5 gm.

One milligram of tropaeolin 00 injected into a frog produced convulsions an hour or more after injection. As little as 0.15 mg. caused violent convulsions and tetanus in from two to forty-five minutes.

Experiments are in progress in which other explanations for the above phenomena than that given by Barbour and Abel are being tested, but for the present no better solution than the inhibition theory can be offered.

In regard to the chemical properties of the four substances, acid fuchsin, phenolsulphonephthalein, naphthol yellow and tropaeolin 00, it is to be noted that they are all dyes soluble in water, that they are all sulphonated bodies, and that they are all taken up by the nervous system, as may be seen by inspection of the brain and spinal cord either directly or after the addition of chemical reagents.

The Action of Drugs on Oxidative Processes. C. Voegtlin. From the Pharmacological Laboratory, the Johns Hopkins University.

Epinephrin, when introduced into the circulation of mammals, is very rapidly destroyed by oxidation. Using a constant inflow of a dilute epinephrin solution, a constant rise in blood pressure is obtained, corresponding to a definite rate of oxidation of epinephrin. By injecting simultaneously with the epinephrin chemical substances which are known to influence the oxidation of epinephrin *in vitro* (acids, alkalies, iodine, etc.), a marked change in the degree of the physiologic action of epinephrin is obtained,

this being made evident by the alteration in blood pressure. In case the tested chemicals do not have any marked effect on normal blood pressure one has good reason to assume that any change of the action of epinephrin is due to an influence on the oxidation of this latter substance. This method, when studied more in detail, may perhaps prove to be of value in testing the action of drugs on biologic oxidation in general.

The Toxic Action of Amianthium Muscaetoxicum. C. L. Alsberg.
From the Bureau of Plant Industry, U. S. Department of Agriculture.

This liliaceous plant contains a solid alkaloid which has not been obtained in crystalline form. The alkaloid is of extreme toxicity, producing death from respiratory paralysis. The effect on the circulation is less prominent than that on the respiratory center. Striped muscle is affected in such a way that fatigue, both by direct and indirect stimulation, is very much more rapid than normally. Relaxation is somewhat delayed, but no distinct veratrine effect was obtained, though the alkaloid in some respects resembles veratrine chemically.

Antagonistic Studies with Alcohol and Caffein. J. D. Pilcher and T. Sollmann. From the Pharmacological Laboratory, Western Reserve University.

Has appeared in no. 3, vol. iii, pp. 267-299, this Journal, as a paper by J. D. Pilcher, entitled Alcohol and Caffein: A Study of Antagonism and Synergism.

Inhibition of Iodide Absorption by Chloride. P. J. Hanzlik and T. Sollmann. From the Pharmacological Laboratory, Western Reserve University.

Appears in full in the present number of this Journal as a paper by P. J. Hanzlik, entitled: Quantitative Studies on the Gastro-intestinal Absorption of Drugs: II. The absorption of Sodium Iodide.

The Advantages of Single over Multiple Doses of Antitoxin and of Intravenous over Subcutaneous or Intramuscular Injections.

William H. Park. From the Division of Laboratories, Department of Health, New York City.

Different investigators have tested the rapidity of absorption of antitoxins from the tissues and drawn attention to the superiority of intravenous injections. Notwithstanding their reports subcutaneous injections are still generally employed in the treatment of both diphtheria and tetanus. It was decided to repeat the experiments of others and add new ones.

A series of rabbits and of goats were injected with 10,000 units of diphtheria antitoxin and bled at intervals for ninety-six hours. A number of persons suffering from diphtheria were examined as to the antitoxin content of the blood during the same period of time. The following two tables give one example of each method of injection in goats and one for each in human beings suffering from diphtheria.

The great advantage of the intravenous injections is manifest.

THE COMPARATIVE VALUE OF SINGLE AND MULTIPLE DOSES

The question of giving the total amount believed to be necessary in a single injection, or dividing it into two or three injections to be given at intervals of six to twelve or more hours, is an important one. The slow absorption of the antitoxin from the tissues would seem to make the single injection by far the better method. The following table shows the antitoxic strength of the blood in the two methods.

It can be seen that the single dose gives much greater antitoxin content for the first three days.

A large series of cases treated by the different methods has shown that the intravenous injections can be safely given. In all cases of tetanus and in severe cases of diphtheria intravenous injections are indicated.

(1) *Antitoxin content of blood in goats injected*

(Each receiving 5000 units)

METHOD OF INJECTION	UNITS IN 1 CC. AT DIFFERENT INTERVALS OF TIME						
	3 h.	6 h.	12 h.	24 h.	48 h.	72 h.	96 h.
Intravenous.....	4.0	3.5	3.0	2.6	2.4	2.0	1.8
Subcutaneous.....	0.1	0.5	1.0	2.5	2.9	3.0	2.9
Intramuscular.....	0.3	1.3	2.0	2.8	2.8	2.6	2.4

Weight of goats about 25 pounds each..

(2) *Antitoxin content of blood in three adult diphtheria patients*

(Each received 10,000 units)

METHOD OF INJECTION	UNITS IN 1 CC. AT INTERVALS OF HOURS						
	3 h.	6 h.	12 h.	24 h.	48 h.	72 h.	96 h.
Intravenous (1).....	3.0	2.7	2.4	2.0	1.5	1.0	0.8
Subcutaneous (2).....	0.1	0.2	0.25	0.4	0.55	0.65	0.7
Intramuscular (3).....	0.2	0.35		0.6	0.6	0.59	0.55

Weight of number 1 about 100 pounds; of number 2 about 110 pounds; of number 3 about 120 pounds.

	UNITS IN BLOOD AT			
	12 h.	24 h.	48 h.	72 h.
Single dose.....	0.25	0.4	0.55	0.65
Dose divided into three given at 12-hour intervals.....	0.08	0.2	0.48	0.62

THE PHARMACOLOGICAL ACTION OF VANADIUM

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CONTENTS

I. Historical note.....	478
(a) Pharmacological investigations.....	478
(b) Vanadiumism.....	479
(c) Tests for vanadium.....	479
II. Symptoms of vanadium poisoning.....	480
(a) Nervous system.....	480
(b) Circulatory system.....	481
(c) Gastrointestinal system.....	481
(d) Respiration.....	482
(e) Blood changes.....	482
(f) Excretion.....	483
(g) Pathological findings.....	484
III. General action.....	485
(a) Alimentary canal.....	485
(b) Lymph flow.....	488
(c) Respiration, oxidations.....	488
(d) Heart.....	491
(e) Vessels, temperature.....	492
(f) Perfusion experiments.....	503
Kidneys.....	504
Spleen.....	504
Intestines.....	504
Leg.....	504
(g) Kidneys, liver.....	506
(h) Central nervous system.....	506
(i) Smooth muscle.....	506
(j) Feeding experiments.....	507
(k) Comparison of ortho- and meta-vanadate of sodium.....	508
IV. Conclusions.....	509
V. Bibliography.....	511

I. PHARMACOLOGICAL INVESTIGATIONS

In the year 1876 John Priestly, Platt physiological scholar at Owens College, Manchester, published an extensive article on the action of vanadium. He observed that the cause of death is central respiratory paralysis. With reference to the circulation he noted a disappearance of the Traube-Hering curves; a fall in blood pressure which he attributed to an action on the vasomotor center and on the heart itself; and that irregularities and final slowing and weakening of the heart beat were due to an action on the "intracardiac nervous mechanism." A rise in blood pressure in rabbits which he observed after section of the cord he attributed to a greater vigor of the heart. He also recorded the presence of congestion and inflammation in the alimentary mucous membrane, and a primary stimulation of the central nervous system followed by depression or paralysis. The size of the lethal dose he placed between 9.18 mgs. and 14.66 mgs. of V_2O_5 per kilogram of body weight for the rabbit.

Gamgee and Larmuth (1876-77) using the metavanadate, the pyrovanadate and the orthovanadate of sodium came to the conclusion that the metal acted in a manner practically analogous to that of digitalis. They, however, appear to have held the opinion that the "intracardiac nervous mechanism" was affected in some way that did not exactly correspond to the effects of digitalis. In the frog they observed that the ventricle finally became contracted from the action of vanadium but that vagus stimulation did not cause a dilatation, and that during perfusion the ventricle became permanently contracted while the auricle still beat.

In 1878, Dowdeswell described an extensive fatty degeneration of the liver which could be produced by prolonged administration of the drug either by way of the alimentary canal or by hypodermic injection.

In 1880-86, the chemists, Witz and Osmond, suggested that the oxidizing properties of the metal might be used therapeutically to accelerate the oxidations of the body. Later this view was eagerly taken up by a large number of French investigators,

notably Lyonnet, Martz and Martin, Hallion and Laran, Berthail, Anceau, LeBlond and David, Weber, Perlemann, Delarue and Helouis, Rodet, Pecourt and others.

Clinically, vanadium has from time to time been used for a great variety of pathological conditions. Among them may be mentioned first and foremost tuberculosis. Other conditions in which its action has been stated to be most beneficial are anemia, chlorosis, syphilis, rheumatism, diabetes, arthritis deformans, heart disease, cachexia, sciatica, neurasthenia, chorea, "dyspepsia," anorexia, myelitis, bronchitis, etc. Aside from these presumably rather general actions, it has been highly recommended as a local antiseptic, especially in general gynecological practice, and in various skin affections.

Vanadiumism. Chronic poisoning can readily be produced in animals. Similar symptoms have also been reported in workers in the steel industries, and in professions where laborers are exposed to the metal. The symptoms are described as consisting of diarrhoea, often followed by marked constipation, anemia, emaciation, loss of appetite, nervous disturbances, etc. The urine may show albumen, casts and blood. The lungs, kidneys, liver and gastro-intestinal tract are reported as showing extensive lesions, those in the lungs generally proving the most serious, and often ending fatally from pulmonary hemorrhages, the immediate cause of which seems to be marked fits of coughing. A persistent, dry, hacking cough appears to be one of the more constant symptoms of chronic vanadium poisoning. The literature on this phase of the subject seems to be very limited.

The following tests which I have had occasion to use a number of times during the course of these experiments may be briefly described here.

I. If a ferrocyanide solution be added to a vanadium solution made acid with hydrochloric acid, a green or yellowish green precipitate is formed. Copper, iron, zinc, uranium and chromates interfere with the test.

II. On the addition of hydrogen peroxide to an acid solution of a vanadium salt, or a solution of vanadic acid, a red color is produced if vanadium be present to the extent of 1 to 30,000. Mix-

tures of ferric and ferrous salts and chromic and chromate salts interfere with the reaction.

III. If a few drops of vanadium solution be added to a small quantity of strong sulphuric acid to which a crystal of strychnine sulphate has been added, there is at first produced a violet color which after a few minutes changes to a rose color. Chromates interfere with the test.

II. SYMPTOMS OF VANADIUM POISONING

The symptoms of acute poisoning resemble rather closely those produced by other irritant metallic substances.

Nervous system. When a large dose is administered subcutaneously to a frog there is at first produced great excitement, agitation and convulsive movements. The animal may leap forward violently and blindly and alight on its back, and perhaps struggle for a time before it can right itself. These symptoms develop immediately upon the injection and at a time when it is obviously entirely too early for the drug to have been absorbed. The only explanation possible is that these results are due solely to the local irritation caused by the administration of the drug. Several writers have described these convulsive symptoms at considerable length, and have been led thereby to compare it to such substances as digitalis or picrotoxin. After the solution really begins to be absorbed and to act on the central nervous system and other structures, there ensues a gradual and progressive depression and paralysis which ends in death.

In mammals a very similar series of symptoms are produced. The early excitement is obviously due to local irritation. In these experiments I have used solutions of the sodium orthovanadate, which are slightly alkaline and this reaction probably has something to do with the results. Previous observers have, however, used such solutions and I have been anxious to verify their observations under corresponding conditions. As the drug is absorbed, and its general systemic action comes on there is produced a gradual progressive depression, manifested by somnolence, weakness and final paralysis. A sort of restless uneasiness

lasting for a few moments at a time is generally observed during the intoxication.

Circulation. The circulation is undoubtedly considerably affected but the results are not readily interpreted in the normal animal. The early excitement causes an increase in the pulse rate, and perhaps a rise in pressure. It is probable that if entirely neutral salts were used these results would mainly be absent.

Aside from these rather superficial effects there are, however, other circulatory phenomena which may be noted from time to time. In some animals the pulse rate is greatly reduced, and often there appears a variation in the rate from time to time. The respiration and nausea indirectly affect the circulation. The temperature has been frequently reported as showing a progressive fall. I have found this to be the case although at least one observer noted an increase in temperature. I shall take this point up again.

Gastrointestinal system. In normal animals it would appear that the principal effects of acute poisoning fall upon the gastrointestinal system. The earliest symptoms manifested in this direction consist in the flowing of saliva, retching and vomiting. The flow of saliva is rather abundant sometimes, but I am inclined to believe it is due entirely to the nausea, although another factor cannot be entirely excluded at present. In etherized animals there also often seems to be an increased flow of tears (greater than ether irritation would produce).

Some fifteen or twenty minutes after hypodermic injection into dogs, a rather marked borborygmus begins. Some time later this is followed by a diarrhoea which in severe cases may be noticeably hemorrhagic. Generally it is not profuse. In fatal cases the diarrhoea generally continues until death (in dogs). I have not observed any marked gastrointestinal symptoms in rats or guinea pigs, the main action of the drug appearing to fall on the central nervous system in these animals, as it does in the frog.

In dogs a moderate degree of intoxication does not seem to produce serious gastrointestinal disturbances. It is, perhaps, worthy of mention in this connection that this gastrointestinal action of the metal may have to be duly considered in any proposed therapeutical utilization of the substance.

In experiments on etherized animals large doses of vanadium increase the peristaltic movements in a manner quite similar in extent to veratrine under like conditions.

Respiration. The final cause of death is respiratory paralysis. This is about the only conclusion in which there seems to be a perfect unanimity of opinion among observers. When normal animals are injected hypodermically with vanadium solutions, the respiration is at first indirectly affected from the pain. After some time the breathing tends to become somewhat irregular, perhaps slower, and dyspnoeic symptoms supervene. Cheyne-Stokes respiration has been reported. I have often seen some indications of this form of respiration. As the intoxication becomes deeper, the animal continues to have more and more difficulty to breathe. After some hours the normal respiratory movements are wholly replaced by deep gasping motions by which life may be prolonged for a considerable time. It appears that the inspiratory phase requires great effort. The cause of this is obscure for the force of the inspiratory muscular movements seems fully as great, and perhaps greater, than in the normal animal. Often a distinct resemblance to the respiratory action of aconitine is observed. It may be noted also that certain other features of the intoxication often resemble aconitine effects. Toward the end the deep gasping movements are obviously markedly influenced by the increasing asphyxia; they finally grow feebler, shallower, less frequent and at last cease, the final termination generally being marked by weak general convulsive movements.

Blood changes. No immediate effects are produced on the blood. The normal bands are seen in the spectrum, and reduction by ammonium sulphide, etc., takes place in a perfectly normal manner. Certain observers have reported that animals dying from acute poisoning with sodium metavanadate do not show normal clotting of the blood, after death. I have not been able to fully verify this observation, and in no case has the blood failed to clot firmly when drawn off after repeated large injections of vanadium. On purely theoretical grounds I am inclined to believe that the clotting time of the blood may be indirectly influenced. The reasons for this will appear in connection with the action

of the metal on the visceral organs, in which certain elements necessary for coagulation appear to be formed.

Various observers have reported that the continued use of small doses for some time will cause an increase in the number of red cells and a rise in the haemoglobin. This has generally been tried out in patients rather than in animals. The general opinions in this respect have led to a comparison of the action of vanadium with that of arsenic. There, however, certainly exist the most striking differences between the pharmacological action of these two substances. The leucocytes have not generally been mentioned as undergoing any very marked variation in the class of experiments and observations usually carried out.

Excretion. Priestly was unable to show that any of the metal was excreted by the kidneys. But he and other observers have shown that it is eliminated by the gastrointestinal canal. I have been able to verify this latter finding in animals which had received large hypodermic injections. I have also obtained faint tests for the substance in the urine, but I have not obtained any traces of the metal in the saliva, vomitus or lachrymal secretion. Dutton (15), however, states that in chronic poisoning in industrial workers, the nasal and lachrymal secretions may contain the element. It seems that the substance is eliminated slowly, for the effects last a long time, and tests of the excretions usually show only weakly positive reactions. It appears that the substance exercises a very definite selective action on certain structures of the body. It does not necessarily follow, however, that most of the substance will become localized in these areas. There seems to be some vague and rather indefinite action exerted upon the nasal and respiratory mucous membranes, and upon the conjunctiva, or some immediately related structures. Possibly this action is due to a slow but continuous accumulation of the substance in these membranes, or to a feeble, prolonged excretion of the metal by the mucous surfaces involved. I have, however, obtained no positive tests in this direction. I have repeatedly observed these symptoms in dogs to which the drug was administered by a stomach tube. The presence of these symptoms in persons exposed to dust, etc., in establishments where vanadium

is used may simply indicate the accumulation of vanadium upon the membranes from the air, but it hardly seems that this is probable in animals which receive solutions into the stomach by means of the tube.

Pathological findings. In acute poisoning the pathological findings are those regularly found following general gastrointestinal irritation. Congestion and hemorrhages are present in the intestines, kidneys, liver, spleen, and sometimes in the lungs. These observations have been verified by many observers. The uterus is occasionally found to show some congestion. Dowdeswell, in 1878, described an extensive fatty degeneration of the liver when an animal was treated for some time with vanadium. Other organs may also be similarly involved.

In rabbits which received intravenously large, rapidly fatal doses (death occurring within five to ten minutes) I have found the left auricle and left ventricle both to be contracted to the utmost limit, and to be very hard and firm, while both chambers of the right heart were rather more soft and flabby than in ordinary conditions. Probably some of the dilatation of the right chambers was due to venous blood which was poured into the heart after it had ceased to empty itself, but this could not account for the whole effect, for the muscular walls of the left heart were firmly contracted and the coronary arteries on that half of the heart were greatly constricted, while those of the right heart were dilated. In death following chronic poisoning in man extensive lesions in the lungs have been mentioned as being among the most marked pathological findings. I have not had an opportunity to personally verify these findings.

Great variations in the size of the lethal dose have been reported by various observers. In one of my experiments a small dog received subcutaneously approximately 80 mgs. per kilogram of body weight and recovered. This is considerably more than several investigators have found to cause death. A small rabbit received 8.1 mgs. per kilogram intravenously and showed very profound symptoms for some time, but after about half an hour exhibited marked signs of recovery. It was then given a second injection intravenously of 16 mgs. per kilogram of body weight

additional, and died in four and one-half minutes. The solutions used in these instances were of the sodium orthovanadate. In all probability much smaller doses of the metavanadate would have been fatal.

III. GENERAL ACTION

Alimentary canal. The action upon the gastrointestinal canal is rather striking. The vomiting which occurs regularly, after the administration of large doses of vanadium to a normal animal, shows the involvement of the digestive organs. The exact nature of the action of the metal on the intestines is obscure, and I have not so far been able to devise any experiment which entirely clears up the situation. There undoubtedly occur the usual localized reactions, which are common to most irritating substances (particularly the irritating metals) in general. This action results in vomiting, retching, diarrhoea, passage of mucus and blood in the feces, etc. The intestinal mucosa shows congestion and hemorrhages after death. Probably this latter lesion is not produced until rather late in the intoxication. In the milder cases of poisoning, blood seldom is seen in the stools.

There is a marked increase in the peristaltic movements. These movements are coördinate and progressive as generally observed in experiments lasting only for a few hours. They are often very marked in etherized animals, and defecation during an experiment is a rather regular occurrence. It appears to closely resemble such bodies as veratrine in this respect. Intravenous administration of the substance is especially liable to cause a marked increase of peristalsis.

The local irritation, both directly and reflexly, probably causes much of the abnormal peristalsis. Aside from this, however, other factors seem to be involved. A graphic record of the action of the metal on the intestines resembles almost exactly the effect shown in a similar tracing obtained by the injection of nicotine. It is to be noted in this case, however, that the vascular changes have much to do in determining the exact appearance of graphic records as usually obtained. It is of some significance that the

peristalsis initiated by vanadium can be at once allayed by the injection of epinephrine (adrenaline).

A further comparison may be obtained by the use of barium, which is generally considered as acting specifically on smooth muscle. When a dilute solution of barium chloride is dropped on a small localized area of the intestine, there is soon produced a very marked anemia and intense contraction, which remains closely restricted to the area treated with the solution. If the experiment be repeated with the substitution of a vanadium solution for the barium, there will be no constriction of the vessels and no contraction of the gut wall. But both of these phenomena occur rather profoundly when the vanadium is injected intravenously. It would appear from this that the vanadium either cannot penetrate the tissues, or that it acts only when carried to the muscle fibers by the blood, or that it acts largely on the nervous structures.

When isolated loops of intestine are excised and perfused outside the body it is found that vanadium added to the perfusion fluid will still cause an increase in the peristaltic movements. And the treatment of such loops with atropine will not prevent the increase of movement due to subsequent injections of vanadium. This observation indicates that the vanadium probably acts directly on the muscle. But the inhibition of peristalsis by adrenalin reminds one of a nervous action. And the failure of the gut to contract when treated locally with vanadium (as occurs under barium) resembles a nervous origin. The general appearance of the movements, as observed following intravenous injections, is of such a nature as would probably lead one to suspect that they were due to some form of direct nervous control. But one would naturally be inclined to infer from the mere nature of the substance (an irritating metallic salt) that its main action would probably be a muscular one rather than that a nervous mechanism would be directly stimulated. The vascular changes should be carefully kept in mind in connection with the peristaltic variations for a very profound constriction which is rather persistent in character occurs in this area.

In chronic poisoning in the human species there appears to be generally produced a diarrhoea for several days or weeks, and then a very marked and obstinate constipation ensues. It seems, however, that this course is not always followed out. One of the distinctive symptoms of chronic lead poisoning is a very obstinate constipation. This is usually associated with a severe colic. Occasionally diarrhoea occurs. The exact action of lead in producing these effects is unknown. Harnack decided that colic thus produced in animals could be relieved by atropine. I have not been able to find any reference to the use of atropine in the human subject in chronic vanadium poisoning, but atropine does not appear to check the intestinal movements induced by vanadium in excised, perfused segments. In dogs which receive daily doses of vanadium for long periods (several months) at a time, there is generally at first produced a rather marked diarrhoea, and this later tends to be replaced by constipation. There seems to be a rather closely marked analogy between the action of lead and that of vanadium on the intestines, and I have observed several other points of resemblance in other directions, particularly with reference to the blood pressure, urine flow, etc.

Considering all the evidence at present available I am inclined to believe that the greater part of the action of vanadium on the intestine is due to an action on the muscular wall, but that there must also be involved some controlling nervous influence which is stimulated to activity by the metal and which probably may continue to act for rather long periods of time, following injections of vanadium.

The salivation in acute poisoning is by no means so abundant as under pilocarpine. It is apparently due mainly, if not entirely, to the nausea and vomiting. I have not obtained any direct evidence of a stimulation of the salivary nervous mechanism.

The vomiting is due to local irritation in the gastrointestinal tract. It seems unlikely that any direct central action is involved. The increased peristaltic movements in the alimentary canal may reflexly help to produce the emesis. This seems not improbable for I could not get a positive test for the metal in the vomitus

(or saliva) of an animal which had been poisoned by subcutaneous injection of the substance.

The secretions of the alimentary canal, which others have examined, do not seem to be markedly affected in any way by small quantities of vanadium.

Lymph flow. In some early experiments I was impressed with the character of the flow of the lymph from the thoracic duct and that from the head region, when both were collected simultaneously in an etherized animal treated with intravenous injections of sodium orthovanadate. It was found that while the head lymph was not appreciably affected either in quantity or quality, that collected from the thoracic duct underwent marked variation.

For some time following each injection there was a very considerable increase in the outflow. This gradually subsided, but after some time a second injection of the drug would renew the outflow. Late in the intoxication there were occasionally observed slight indications of blood in the collected lymph. This, however, was never marked. The variation in rate of outflow first induced me to examine more closely into the action of the metal on the visceral organs. It appears that the increase in thoracic lymph flow following each injection is due to the increase of intestinal movements, to changes in the calibre of the visceral vessels, and to a rise in general blood pressure. Probably this action on lymph flow might have some slight influence on any therapeutical advantages accruing from the use of the substance. Tests made for vanadium in the collected lymph were negative, but as these tests are not highly sensitive, small quantities of the metal may have been (and probably were) in the lymph even if no reaction was obtained to indicate its presence.

Respiration and oxidation. The respiration is very markedly affected by vanadium. The action appears to be due to a direct central influence. When large doses are administered a marked dyspnoea appears early. I have suspected several times that some constriction of the bronchial musculature may take place and thereby hamper the respiration, but I have not as yet satisfactorily demonstrated this in an experiment. On auscultation

tion the breath sounds of the dog are harsh, but injection of vanadium in moderate doses appears not to appreciably modify their character. The dyspnoea under toxic doses often reminds one of that produced by aconitine, but it is not so irregular in the early stages of the poisoning as is the case with aconite. Late in the intoxication with vanadium the animal breathes only by shallow, infrequent gasps. These grow gradually more and more feeble and finally cease, a few weak asphyxial convulsions generally marking the termination of the intoxication. The heart beat persists until after paralysis of the respiration.

Witz and Osmond early called attention to the possibility of an oxidizing action which they conceived vanadium salts might have in the body. Many clinicians (mostly French) took up this idea and from it a considerable number of preparations, which are now on the market, have arisen. According to this notion, vanadium plays the part of an oxygen carrier. It is described as deriving oxygen from the oxyhaemoglobin (by which, for example, vanadic acid, or pervanadic acid, is formed in the tissues) and then passing, or rather forcing, this oxygen on to the tissues. (A reduction of the vanadic, or pervanadic, to the hypovanadic acid thus follows. This in turn seizes oxygen from the oxyhaemoglobin and vanadic acid is thus re-formed.)

The marked instability of certain vanadium combinations and its well known powers of producing chemical oxidations outside the body seem to have first led to this conception of its action in the animal body. It thus appears to "increase the oxidations of the body." As proof of this action the evidence seems to be that there have been noted, clinically, an increase of urine flow, a rise in the urea excretion and a decrease in uric acid output, an increase in appetite, strength and weight, and general brightening up of the patient's mental outlook. From time to time confirmation of many of these observations has also been reported from animal experiments. It has also been claimed to decrease the urinary sugar output in diabetes. It has been claimed that very minute quantities of the substance are sufficient to cause a great increase in the tissue exchanges, and the activity has thus been compared to a ferment action, in fact a sort of peroxidase

reaction. And inasmuch as the vanadium is described as carrying out a reversible process, so far as the metal itself is concerned, it would seem that we have to deal here with what one American writer has called a sort of "perpetual motion" regenerator of the vitalities of the organism. On the basis of these alternate oxidations and reductions a comparison has been made between the action of vanadium and that of arsenic and arsenious acids as described by Binz and Schulz.

This view of the oxidative powers of vanadium has not, however, passed unchallenged. It is rather difficult to devise a set of experiments which will definitely decide a point of this nature. Professor Soulier was inclined to question whether or not the vanadium might just as readily take oxygen from the cells as from the haemoglobin. Lyonnet, Guinard, Martz and Martin¹ who believed the main action of the drug rested on its oxidative powers, studied the respiratory output of carbon dioxide in guinea pigs treated by the subcutaneous administration of vanadium. Their results varied, for they "sometimes obtained an increase and sometimes a decrease" in the amount of CO₂ exhaled.

I have attempted to determine the effect of vanadium on the total pulmonary ventilation by means of a spirometer, by which the volume of expired air per minute was carefully measured both before and after the administration of vanadium. The animals were etherized and a blood pressure tracing was taken from the right carotid artery. The blood pressure was carefully observed in order to keep a check on the animal's condition. A considerable number of determinations were made in order to establish the normal rate of respiration, and then a very small dose of the drug (sodium orthovanadate) was injected into the left femoral vein. Observations were then recorded for a time, and after several minutes another injection was made and more observations were recorded. In this manner the effect of very small, medium and large doses were studied on each animal.

On the whole the results were not very conclusive. It is difficult to control the degree of anaesthesia, and slight variations

¹ Soc. Biol., 1899, 22 Juillet, p. 708.

in the narcosis produce immediate results in the respiratory readings. It is only by averaging the readings for a large number of determinations that these errors can be approximately corrected. It seems that with small doses such as would have to be used therapeutically, the results are very variable. Perhaps in over half the cases a slight increase in the total amount of air passing through the lungs is shown. And in some cases this undoubtedly occurs. I am inclined to doubt very much, however, that this is any definite indication that the drug has increased the metabolism of the body by its oxidizing powers. For much more striking results in this direction may be obtained with many other drugs, e.g., atropine or apomorphine. And further, vanadium has marked specific actions in other directions which may quite readily account for most of the phenomena which have been attributed to it on the basis of its oxidizing powers. And it seems very evident that most of the workers who have attempted to utilize the metal therapeutically, were unaware of the main action of the drug. A complicating phenomenon which must be considered in relation to all such experiments as ordinarily performed is the fall in temperature which follows the injection of vanadium. This factor undoubtedly would influence the respiration and consequently neither carbon dioxide output nor total pulmonary ventilation could throw much light on the oxidizing powers of the substance in the tissues.

Heart. The action on the heart has been the subject of much discussion in the literature. The older writers seemed to believe that some "intrinsic nervous mechanism" was directly influenced by the metal. A little later the notion became established that vanadium acted very much in the same manner as digitalis, strengthening the heart muscle, and slowing its beat from central vagus stimulation. This view appears to be the one generally quoted at the present. Lyonnet, Martz and Martin found the cardiovascular system but little influenced, while Hallion and Laran, and Luzzatto found that the substance produced a rise in pressure. Hallion and Laran observed a marked irregularity of the heart in animals treated by injections of vanadium. On section of the vagi nerves this irregularity at once ceased, and

they, therefore, attributed it to a strong medullary stimulation. This they also appear to have considered ample evidence that the marked rise in pressure followed an analogous stimulation of the vaso-constrictor center. Most observers, in studying the cardiac action of the substance appear to have used frogs for their experiments. In the mammalian heart, however, the digitalis action appears to be very much less, in fact in many cases the sole action on the heart seems to be a slowing of its rhythm. With moderate doses, tracings taken directly from the exposed heart (dog) usually show but little change in the amplitude of the beat with perhaps a little increase in diastole, and a corresponding decrease in systole. Probably this effect on systole and diastole is a direct result of the general rise in blood pressure in which the heart appears not to take any prominent part. The slowing of the mammalian heart is usually partly due to vagus action for section of both vagi may accelerate the beat. But this does not seem to always be the case for slowing sometimes occurs with approximately the same sized doses of vanadium after section of the vagi, or injection of atropine. And a small injection of epinephrine will at once greatly stimulate and accelerate a heart which has been slowed by vanadium. It appears, therefore, that the action of the metal must be directly on the heart muscle. It may further be noted that the extreme contraction of the left chambers of the heart seen in rabbits, which are rapidly killed by the intravenous injection of large doses of vanadium, indicates a direct action on the heart tissue itself.

In ordinary therapeutic doses it does not seem probable that this cardiac action could be very great, and in all likelihood would not generally be noticed at all. This point, however, cannot be fully determined by animal experiments alone.

Vessels, temperature. It is on the vessels that the most striking action of vanadium is observed. The general blood pressure is rapidly raised and maintained at a higher level for some time. This has long been explained as due to a strong stimulation of the bulbar cardiovascular centers. Hallion and Laran believed they had found amply justification for this view, when after section of the vagi nerves the heart which had become irregular

following injections of vanadium at once became regular and showed its normal beat. They found the blood pressure was elevated at the same time, and by analogy they decided that the bulbar vasoconstrictor center was also stimulated. This view of the action of the drug coincided quite closely with that regarding the action of digitalis. Lyonnet, Martz and Martin, whose work has been extensively quoted, failed to obtain any marked rise in blood pressure, and were of the opinion that the cardiovascular system was not greatly influenced by the drug. It has been stated that the excitability of the vagus nerve is depressed by the substance. I have not noted any such results, but electrical stimulation of the vagus trunk slows or stops the heart throughout the whole course of the intoxication in the dog.

When the volume of the kidney, spleen or an intestinal loop is recorded in an animal which is injected intravenously with a solution of sodium vanadate, a most profound constriction of the organ or intestine is observed. It appears that Hallion and Laran also observed a shrinkage in the kidney, and sometimes of other organs. They attributed this result to a strong central bulbar stimulation.

During the course of an experiment one day I was impressed with the great resemblance which a tracing I obtained bore to the action of nicotine under similar conditions. This led me to make a further examination of the vascular action of the metal. I accordingly etherized an animal and arranged to record its blood pressure, respiration and kidney volume. The cord was then severed within the body of the fifth cervical vertebra. The respiration ceased for a little while then became wholly abdominal, showing the action of the phrenics. The blood pressure fell at once to a level far below the normal and as soon as the animal had regained its equilibrium a dose of sodium orthovanadate was injected into the femoral vein. The blood pressure rose in practically the normal manner, and to approximately the usual extent as observed in an intact animal. At the same time a very great constriction of the kidney occurred (Fig. 1). This made it clear that the rise in pressure and renal constriction could not be of central origin. In order to still further verify the obser-

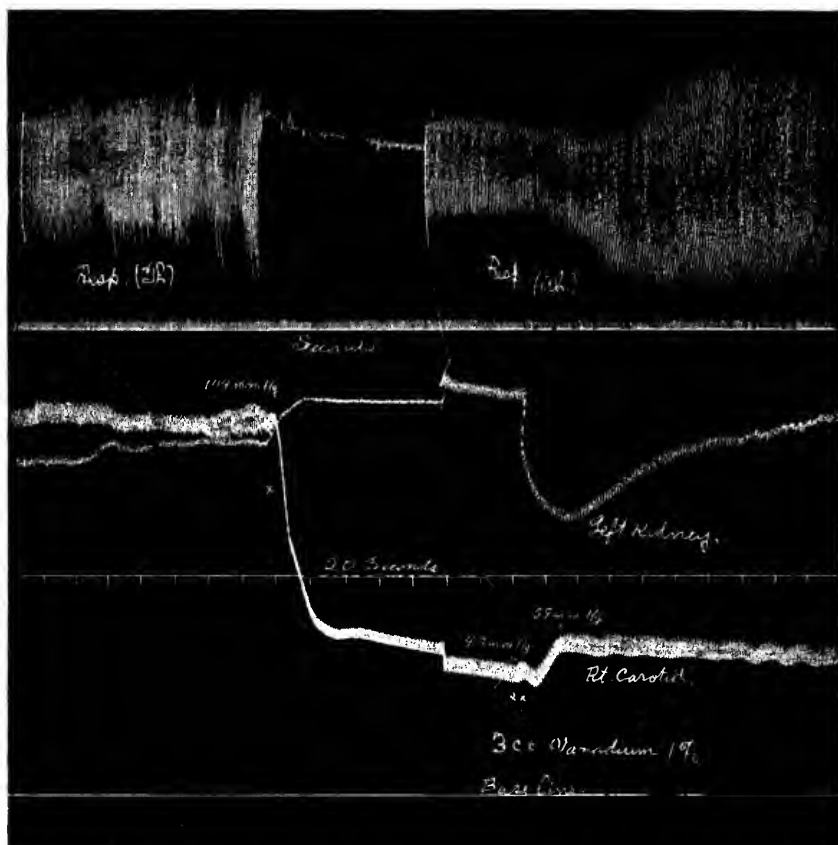


FIG. 1. KIDNEY VOLUME, RESPIRATORY AND BLOOD PRESSURE TRACINGS FROM A DOG

At the point marked "x" the spinal cord was divided within the body of the fifth cervical vertebra. The carotid pressure fell to 48 mm. of mercury, the kidney volume slightly increased and the respiration ceased for a short time from the shock of the operation but soon returned, being then wholly diaphragmatic. Injection of sodium orthovanadate then caused a moderate rise in blood pressure and a marked contraction of the kidney volume. This proves that the medullary centers have little or nothing to do with the ordinary vascular action of the metal, for the rise in blood pressure and shrinkage in kidney volume are practically identically the same as they would have been in the intact animal. This can be even more clearly demonstrated in the decapitated animal.

vation, however, a number of other experiments were performed in which the entire head was removed from the animal and blood pressure and kidney volume recorded. These experiments completely verified the former observation, the vascular action (kidney volume, slowing of the heart, and general rise in pressure) being practically identically the same in the decapitated animal as in the normal intact organism.

The resemblance of vanadium tracings to those obtained by the injection of nicotine led me to suspect an action on some peripheral nervous mechanism. In order to study this point a long series of experiments were performed, a number of the results of which will be more fully discussed in a later section. It will suffice at present to state that no certainly positive proof of any nervous action has so far been demonstrated. But on the other hand, no definite proof has been obtained to show that such a nervous mechanism is not brought into activity.

It has been mentioned in the literature that the Traube-Hering curves disappear after injections of vanadium. I have repeatedly observed these phenomena until an extremely late stage in the intoxication. These curves may, however, be greatly influenced from an indirect cause. For certain results obtained by the use of the oncometer undoubtedly point to a marked peripheral influence upon the general blood pressure. And vanadium appears to have actions in this direction which, so far as I have been able to determine, have not been described in connection with any other drug. In the normal intact animal the final fall of blood pressure is due to heart weakness and central paralysis.

In proportion to the rise in general blood pressure, the shrinkage in volume of the kidney, spleen or intestinal loop under the influence of vanadium is by far greater than that produced by any other drug, with the action of which I am acquainted. A comparison with the active principle of the suprarenal glands may serve to make this clear. When a small dose of this substance is injected, there will be produced a small rise in blood pressure and a small shrinkage of kidney volume. Let us suppose, for example, that the rise in blood pressure produced by a given dose of epinephrine in a medium sized dog amounts to 25 mm. of

mercury, and that the kidney volume is recorded on the drum by means of air transmission from a good oncometer to a fairly sensitive recording tambour. Then if, following the administration of the epinephrine, a dose of vanadium sufficient to give an equal rise in general blood pressure (25 mm.) is injected intravenously the shrinkage in kidney volume will be vastly greater than it was under the epinephrine. This holds good for the kidney, spleen and intestine. I have not tested out any other of the visceral organs. It should be noted, in regard to this experiment, that the rise in general blood pressure produced by vanadium soon reaches its maximum limit, i.e., a rise of 40 or 50 mm., depending on the animal (dogs) will be the maximum which the drug will produce in a given animal, no matter how large a dose may be injected after the maximum height of pressure has been reached. This is very much more limited than is the case with epinephrine. This phenomenon, which has been observed by others, has so far proved very perplexing, for it stands to reason that if a small dose of vanadium will give a slight rise in general blood pressure, then a very much greater dose should give a correspondingly greater rise, as in general is the case with epinephrine. The reason for this, I believe, I have been able to demonstrate in a fairly satisfactory manner. Epinephrine acts on the vessels of nearly all the regions of the body. But these appear to vary somewhat in their sensitiveness and response to the drug, so that when the maximum contraction is approximately reached in one area, there will be other areas still capable of responding if the dose be increased in quantity. Apparently this latitude for action is very much more limited for vanadium than for adrenaline. For while the vaso-constriction of the abdominal organs appears to be possibly more profound than can be produced by adrenaline, the other regions of the body seem to respond but little to the action of the metal. And the maximum limit of contraction in the visceral organs is soon reached, and with comparatively small doses of the metal. Larger doses then do not seem to have any very marked effect on the general blood pressure. If one carefully records the volume of the kidney (Fig. 2) or spleen (Fig. 3), and at the same time takes

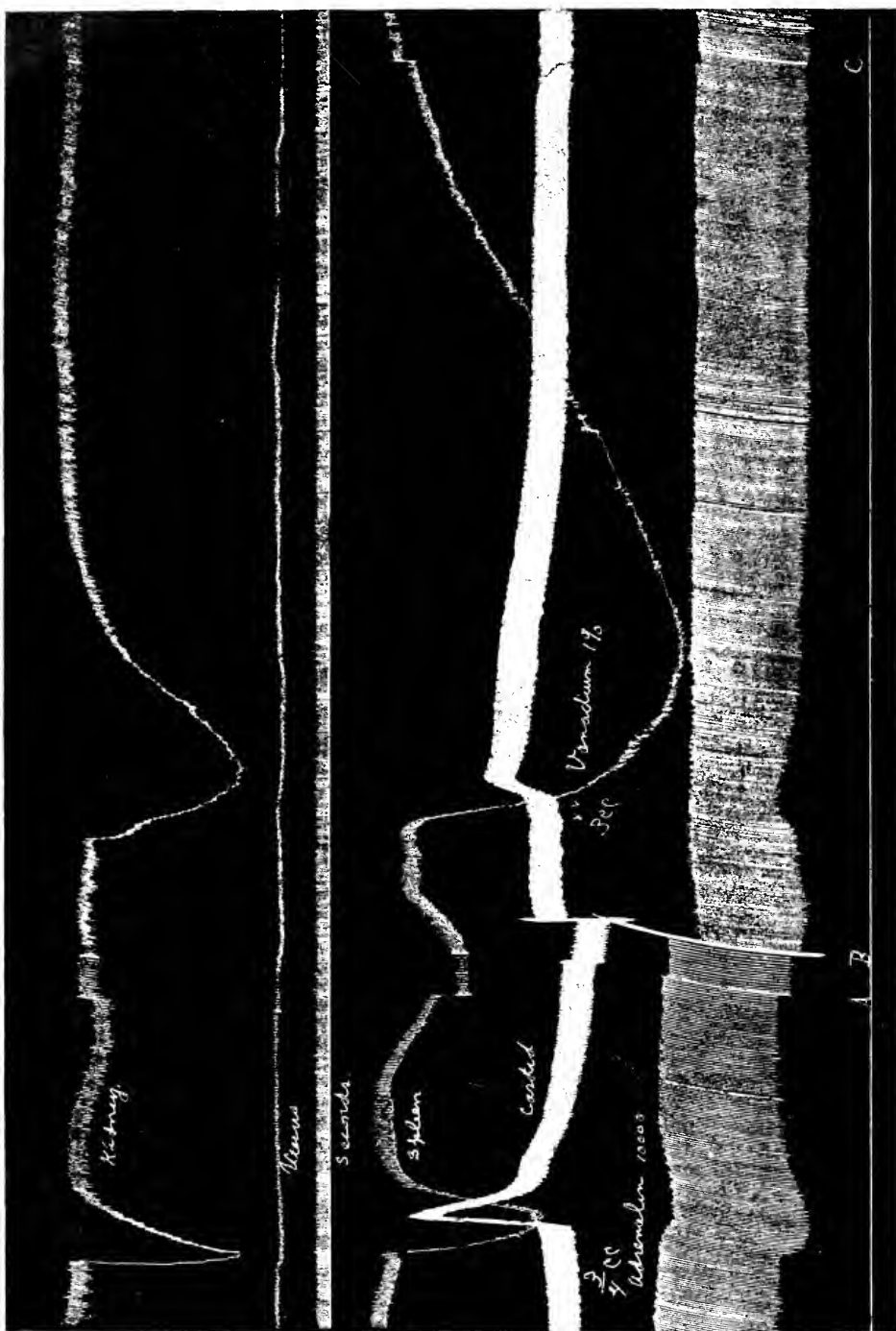


FIG. 3. KIDNEY VOLUME, UTERUS, SPLEEN VOLUME, RIGHT CAROTID PRESSURE AND RESPIRATORY TRACINGS FROM A DOG

Descent of the kidney and spleen tracings indicates contraction of the volume of those organs. The uterus showed no contractions. The adrenal tracing is for contrast and comparison. The spleen contraction is very marked and prolonged. The action

plethysmographic tracings of one of the hind limbs (Fig. 2) of an animal, it will be observed that an injection of vanadium causes a constriction of the visceral organs but an increase in the volume of the leg. This does not mean that an active dilatation of the limb vessels occurs, but that a passive increase in volume is produced by the marked abdominal displacement of blood. If the volume of the leg, which has been removed from the animal, and perfused with Ringer's solution be recorded, it will be found that the addition of vanadium to the perfusing solution will cause a small constriction of the limb. This shows that the metal tends to constrict the muscular vessels as well as those of the viscera, but the effects on the latter are so vastly much greater that in the normal intact animal probably no constriction at all of the former would be produced by administration of the substance. This action might seem to be a fair basis for its use in hemorrhages of the kidney, spleen or intestine. But in regard to the latter it should be remembered that the body also increases the peristaltic movements. And possibly some such action occurs in the kidney and spleen.

A comparison of the action of vanadium with that of barium on the vascular system may serve to explain some of the actions of the former. If the general blood pressure and the kidney volume both be recorded in an animal, and a moderate dose of barium chloride be injected intravenously, there will be produced a marked rise in blood pressure and a small decrease in kidney volume. If, following this result, a moderate dose of vanadium be injected, there will be produced a rise in blood pressure which rather closely resembles the former, but the decrease in kidney volume will be very vastly greater in extent than was produced with the barium (Fig. 4). Barium has long been described as acting directly on the musculature of the heart and blood vessels. The comparative effects of these two substances on the circulation in this case would seem to indicate that vanadium either possesses a very much greater action on the visceral vessels, or else that this substance brings into action some nervous mechanism. It is probable that most of the increase in general vascular tension under barium is due to direct stimulation of the heart,

and that the vessels themselves are much less affected. With vanadium, however, the process must be very different. For the heart is but little (if indeed at all) stimulated while the visceral vessels are greatly constricted and the remaining vessels of the body, while perhaps tending to constrict, may really be slightly dilated. It will be recalled that a very similar difference in action between these two bodies was described in connection

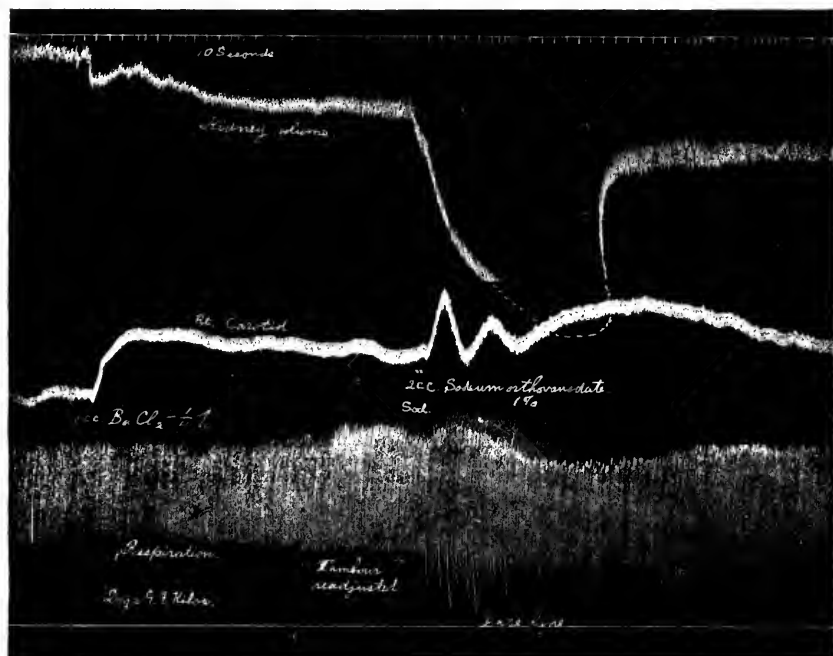


FIG. 4. KIDNEY VOLUME, BLOOD PRESSURE AND RESPIRATORY TRACINGS FROM A NORMAL ETHERIZED DOG

Injection of barium chloride produces a rise in blood pressure and a slight contraction of the kidney volume. Later an injection of sodium orthovanadate gives a similar rise in pressure but a very marked shrinkage in kidney volume. Inspiration = downstroke. The lever of the kidney tambour fell to the cup of the instrument and marked a straight line. A small amount of air or fluid was thus probably aspirated into the oncometer or tambour and tubing. Consequently the recorded ascent of the tracing later probably appears more rapid than it should. This tracing shows the relative cardiac and vascular action of barium and vanadium.

with the intestinal movements. On the basis of these experiments it cannot be decided whether vanadium acts on the muscle fibers, on the nervous mechanisms, or on both.

In order to test this point still further, an attempt was made to utilize the action of apocodeine. But the results were not at all conclusive, and consequently could not be considered. Further experiments in this direction are now being carried out with ergotoxine.

Solutions of sodium ortho- or metavanadate are slightly alkaline in reaction. It seemed possible that this alkalinity might exercise some effect on the general action of the solutions. In order to determine this point a solution of sodium hydrate of approximately the same strength of alkalinity was injected into an animal in about the same sized doses as were used of vanadium. The pure alkali solution produced but very little effect while the vanadium solution was highly active. Incidentally it may be mentioned that careful tests demonstrated that the vanadium solutions used for intravenous injections do not produce intravascular precipitation.

It appears that vanadium exercises a sort of strict selective action for the vessels of the abdominal viscera. This indicates in a way, a nervous action, for the visceral vessels respond more readily than those of most other regions of the body to nervous influences. It must, however, be borne in mind that these vessels may also be more responsive to strictly muscular poisons.

It seems that the metal may be largely excreted by the intestines and perhaps by the kidneys. Possibly the tissues of these organs seize upon the vanadium circulating in the blood and tend to hold it in the rather limited area represented by these organs, in order that it may be more readily excreted. In that case one must remember that the spleen is also profoundly contracted in volume by the metal, and this organ probably takes no part in the excretion of the substance.

Temperature. It has often been observed in animal experiments that the temperature may fall suddenly following an injection of vanadium. It has also been repeatedly reported that the fever of patients treated with the substance undergoes a reduction. Weber

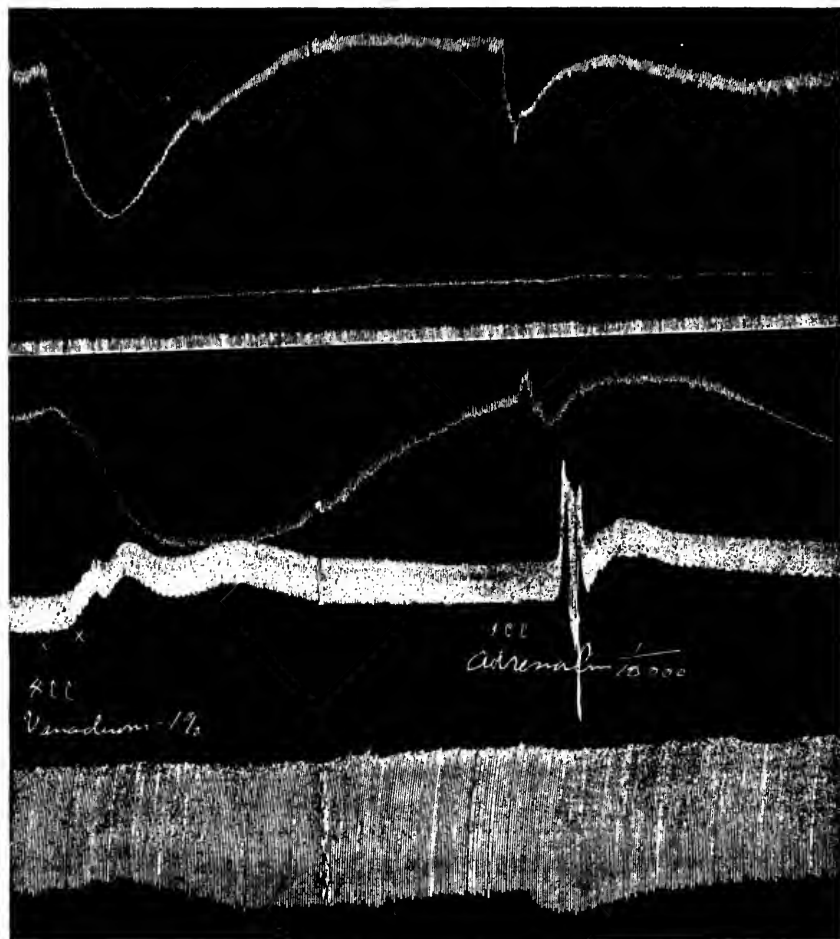


FIG. 5. READING FROM TOP TO BOTTOM, KIDNEY VOLUME, UTERUS, TIME IN SECONDS, SPLEEN VOLUME, RIGHT CAROTID PRESSURE AND RESPIRATORY TRACINGS FROM A DOG

The animal had previously received several injections of sodium orthovanadate. At "x, x," another injection of vanadium was given. The rise in blood pressure is much slower and less in extent than occurred at first. The heart is considerably slowed as shown by the increased amplitude of the manometer tracing. The spleen and kidney still contract markedly but very much less than at the first injection. Later adrenaline was injected. The contraction of the kidney and spleen is very small, while the rise in pressure is almost wholly overcome by slowing of the heart. This shows that repeated injections of vanadium counteract the action of epinephrine.

believed that he observed a rise in temperature under the latter conditions but Delarue hastened to contradict him on this point. In my own experiments I have confirmed the reduction of temperature. So far no explanation has been accepted for this phenomenon. Weber compared the metal to tuberculin and accounted for the rise in fever in the same way as it might follow the use of tuberculin. Delarue objected to this explanation on the basis that if the vanadium cured or ameliorated the tuberculous patients then the metal acted not after the manner of tuberculin but rather as an anti-tuberculin. And he suspected that the "vanadine" or other vanadium preparations acted by destroying in some way the toxins produced in the disease. It appears that he would accept this as an explanation for the reduction in temperature which he observed.

Personally I am not inclined to give much weight to either of these arguments. For the marked constriction of the visceral vessels and the corresponding dilation of those of the limbs and skin which is shown in Fig. 2 seems to be a sufficient cause to produce a lowering of the temperature, simply by increased heat loss at the surface of the body. There can be but little doubt that some of the clinicians have elicited this action in their patients, for Weber himself found the drug contraindicated in arteriosclerosis because the rise in pressure was liable to cause disastrous results. The fact that he noted a rise in pressure seems very good evidence that he had obtained a constriction of the visceral organs.

Perfusion experiments. In order to determine the action of the metal on the vessels themselves a series of experiments were performed in which excised organs were perfused with Ringer's solution to which whipped blood was sometimes added. A perfusion apparatus was arranged in such a manner that the flow of the solution into the artery of the organ was interrupted at regular intervals by means of a water motor, the rate of the interruption being about the same as that of the rate of heart beat. The solution was heated in a tube which passed through a warm water bath. The pressure under which the solution entered the artery was regulated by raising or lowering the pressure bottle hold-

ing the solution. The excised organ or limb was placed inside an airtight vessel, either a small tin box closed by a broad rubber band through which the cannulas passed (a notch being cut out of the side of the box), or into a bottle or jar each of which was closed by a cork or board through which the cannulas passed. The interior cavity of the box or other vessel holding the organ was connected by means of rubber tubing to a recording tambour which marked upon the revolving drum. Injections of drugs were made by means of a hypodermic syringe the point of which was passed into the tube leading into the artery. In this way the volume changes of the perfused organ could be at once recorded on the drum. It was found that the addition of a small amount of vanadium to the perfusion fluid caused a very great decrease in volume of the kidney, spleen (Fig. 6), or an isolated loop of intestine, both the ends of which were firmly ligated. The volume of a perfused hind limb of the dog was also found to undergo some decrease, but the extent was much less in this case than with the other organs. These experiments leave no room for doubt that the main action of the drug consists in a direct peripheral contraction of the vessels, mainly of the abdominal area. A comparison of the relative powers of vanadium and of barium to cause this constriction shows that vanadium is vastly more active than the other substance. It appears also to be able to fully equal, and possibly exceed, the power of epinephrine (adrenaline) to cause shrinkage in volume of the perfused organ. The general contour and the extreme extent and rapidity of descent of the curve of constriction of the organ gives one an immediate impression that we are dealing with an action which must be at least partly of nervous origin. But the apocodeine experiments indicated that it probably occurs after that drug in the intact animal. And a few preliminary experiments indicate that constriction also likely occurs after ergotoxine. If barium is taken as the typical example of a drug acting directly on the muscle fibers of the arterioles, then vanadium must either be a very much more active body in stimulating the muscle fibers of the abdominal vessels, or else the metal must stimulate some nervous mechanism. It is not possible at present to decide which of these

possibilities actually represents the truth. The solution of this problem may be of considerable importance clinically. The rate of outflow from the veins of perfused organs follows the volume changes quite closely. It has been noted before that solutions of the sodium ortho- or metavanadate are slightly alkaline. In order to determine whether or not the constriction of the organs in these perfusion experiments was due to the alkalinity, the solutions used for perfusion were carefully neutralized with tartaric acid in one case and with acetic acid in another. In both cases the action of the vanadium was practically identical with that

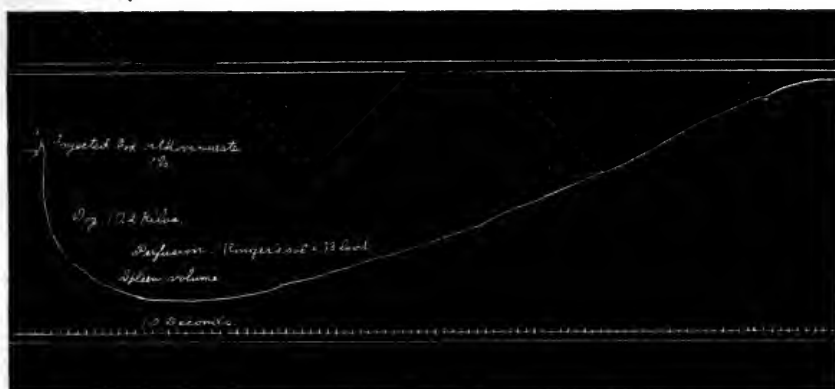


FIG. 6. VOLUME OF AN EXCISED PERFUSED SPLEEN FROM A DOG

At "x" in the tracing sodium orthovanadate solution (1 per cent) was added to the perfusing fluid (Ringer's solution plus whipped blood). A marked and prolonged contraction of the spleen volume followed. This shows the peripheral action of the metal. For method of perfusing see text.

of the unneutralized solution. Evidently the slight alkalinity of the pure salts has but little influence on their specific action.

Kidneys. Clinically it has been observed that the urine flow is increased following the administration of vanadium preparations. This seems to occur rather regularly also in animals which are treated with small daily doses of the metal. The cause appears to be a sort of mild general stimulation of the visceral vessels, and perhaps especially those of the kidney. It is possible that the metal may also stimulate the renal epithelium, either

directly by local irritation, or through some nervous influence. I am inclined to believe, however, that the vascular changes are the main, if not the sole, cause of any variation in amount of urine secreted. In experiments with etherized animals, it appears that all urine secretion is stopped when moderate doses are injected intravenously. The great renal vaso-constriction seems to be the explanation of this. Albuminuria may be produced by toxic doses. The pathological findings in the kidney have already been mentioned.

Liver. I have not performed any experiments on the liver. It is to be presumed, however, that the metal may exercise some influence upon the organ. The vascular changes found after death correspond quite closely with those found in the kidney and intestine, and by analogy one would suspect that some similar action was carried out on the liver. The metal may be partly excreted into the bile, but I have made no tests for its presence here. The clinical reports of changes in the relative amounts of urea (increase) and uric acid (decrease) in the urine may be partly dependent on changes produced by the metal in the liver. But this hepatic action may be wholly indirect in origin, and possibly dependent on vascular changes.

Central nervous system. It would appear that with moderate doses the central nervous system is not greatly influenced. The respiratory center seems to be the most susceptible area. The dyspnoea and final respiratory failure are apparently of central origin. I have not observed any evidence of direct stimulation of any part of the central nervous system with the possible exception of the cardio-inhibitory center. This may be slightly stimulated, but the little acceleration of the heart which follows section of the vagi is probably not noticeably in excess of that produced in this manner in the normal animal. With large, but not fatal doses, consciousness seems not to be greatly affected. But with very large quantities a marked depression and somnolent condition is soon produced. The salivation and emesis probably do not depend on direct central stimulation.

Smooth muscle. The action on the intestine and vessels has already been described. The kidney and spleen show, at some

stage in the stimulation and in practically all cases, a peculiar phenomena which consists in a sort of peristaltic motion. The intestine may also show this, but here the true peristalsis of the digestive movements may be concerned. The normal rhythmic movements of the spleen may also be involved in some of the cases. But it is perhaps in the kidney that this phenomenon shows best. It comes on usually some time after an intravenous injection has been made. It consists of a peculiar irregular alternate constriction and dilatation of the volume of the organ. It seems to be due to alternate contractions and relaxations of the vessels, for it may occur in the excised and perfused kidney.

I have repeatedly attempted to observe the action on the uterus. But in no instance have I seen the faintest indication of a contraction (Fig. 3). It may be that in the late stages of gestation contractions would occur, but in the early stages or in the nonpregnant condition it appears that the uterus is entirely unaffected by the metal (in dogs). The pupil does not seem to be affected unless indirectly from excitement, etc. Stimulation of the sympathetic fibers causes dilatation throughout the whole course of the intoxication.

Feeding experiments. A long series of experiments have been carried out in this line, but the results have not been wholly satisfactory. I shall therefore be extremely guarded in any conclusions which I may draw from them. It seems that this has been the favorite mode of experimentation with most workers in the past. Many observers have found that daily administration of small doses to animals caused an increase in the appetite, weight and strength of the animal. It seems that this occurs in animals which are perfectly normal at the beginning of the experiment. There is an increase in the amount of urine excreted. It has also been claimed that the number of red cells is increased in the blood. A number of these observations I have been able to verify in my own experiments. When larger doses are given there is produced a loss in weight, anorexia, weakness, etc. I have noted that a rather marked diarrhoea usually persists for some time and then seems to cease. A greenish-yellow pus usually appears about the eyes. Vomiting may be present, and occasion-

ally slight indications of pulmonary involvement. I have not found peripheral neuritis to be produced by the substance. But sometimes the animals draw themselves up in a manner which one would suspect indicated the presence of colic. The fatty degenerations of the internal organs, as observed by others, have been mentioned in a previous section.

I have not yet been able to fully complete my observations on the chronic changes in the blood. Numerous smear preparations have been made from day to day from the blood of the animals. In many instances these smears undoubtedly show abnormal cells in the blood. But these variations are so common in the dogs obtained here that one cannot estimate the value of such findings. It seems that after some time nucleated red cells begin to appear in the blood. The cause of these conditions is obscure. I have suspected that this might result from hemorrhages into the intestine, but tests for occult blood in the feces seem to pretty generally be negative, at least unless a marked diarrhoea be present. I have not observed any stippling of the red cells as occurs in chronic lead poisoning.

The metal does not seem to be very poisonous, for average sized dogs may readily take 9 or 10 mm. of the chloride or orthovanadate six days a week, and live indefinitely if properly cared for. This observation may be of value in case the drug should be used therapeutically. The action, however, seems to be very much less marked when the metal is given by stomach instead of hypodermically or intravenously. But, as was mentioned in a previous section, its specific action may be elicited when the drug is given by way of the alimentary canal.

Comparison of ortho- and metavanadate of sodium. Larmuth determined that of the sodium vanadates the least toxic is ortho- (Na_3VO_4), the next more poisonous the meta- (NaVO_3), and the most poisonous the pyro-vanadate ($\text{Na}_4\text{V}_2\text{O}_7$). I have used only the first two of these salts and have very generally verified Larmuth's observations. A considerable discussion has been carried on in the literature regarding the reliability of the commercial preparations of these salts. I have tried a number of samples and found practically no variation among them. I have,

however, preferred to use the recently imported Kahlbaum salts when possible. Fresh solutions were always made up within a few minutes of the time when injections were to be made. I have not found other salts of the metal nearly so satisfactory for experimental purposes as the sodium ortho- or metavanadate. The pyrovanadate is not easily obtained in the market.

In the long list of diseases in which the metal has been reported to do good, one might search a long time before he could find any common factor, the correction of which might be expected to cure or greatly alleviate the malady. Among this list perhaps ought to be mentioned tuberculosis, anemia, chlorosis, lues, rheumatism, diabetes, anthritis deformans, "heart disease," cachexia, autointoxication, sciatica, dropsy, neurasthenia, myelitis, chorea, "dyspepsia," anorexia, bronchitis, skin affections, etc. In addition may be mentioned its use as a local antiseptic in skin and venereal diseases.

Sofar as I can deduce from the literature and from my own experiments I should say that probably the only actions of the metal which may be at all utilized in the treatment of pathological conditions are those which are exerted on the abdominal organs. These consist of a marked localized peripheral constriction, and perhaps toning up of the vessels of the splanchnic area; an increase in the peristaltic movements; and apparently an increase in the urine flow. This latter is probably due mainly to the vascular action.

IV. CONCLUSIONS

1. When administered intravenously the chief action of vanadium is expended on the vascular system. The central nervous system has but little influence on this action, for the rise in blood pressure produced by injection of vanadium into an animal whose head has been removed from the body is almost identical, both in character and extent, with the rise produced by injection of the metal into a normal (etherized) animal.

2. With ordinary doses the mammalian heart is but little affected. The vagus endings in the heart remain active throughout the whole course of the intoxication in the intact animal. Betrachian and

chelonian hearts seem to be more directly affected by the element than is the mammalian heart.

3. An intense peripheral vasoconstriction is produced by the metal in the spleen, kidneys and intestines. In the intact animal the cutaneous and muscular vessels dilate from visceral displacement of the blood, but in perfusion experiments the limb volume also decreases slightly under the action of vanadium.

4. The view held previously that the rise in general blood pressure was due to a strong stimulation of the medullary vasoconstrictor center is wholly wrong.

5. The peripheral constriction is due to a localized action within the organs themselves. It occurs in a perfectly normal manner in the excised and perfused organs of animals, whose general blood pressure has previously fallen to zero under large doses of the metal.

6. With repeated intravenous injections of the same sized doses into an intact animal the rise in blood pressure following each injection regularly decreases until at length a fall will be produced by each injection. This is due first to weakening and paralysis of the vasoconstrictor center and second to a direct depression of the heart.

7. With a moderate dose the maximum rise in blood pressure will be produced and a further increase in the size of the dose will not give any greater rise in the pressure. This seems due to the fact that a moderate dose gives the maximum contraction of the visceral vessels, and a larger dose does not produce any corresponding constriction of the remaining vessels of the body.

8. The peripheral action of vanadium on the visceral vessels is very much greater than that of barium.

9. With doses of epinephrine and of vanadium so adjusted that each will give the same rise in general blood pressure, the vasoconstriction in the kidney, spleen, and intestine produced by vanadium will be very much greater in extent and duration than that produced by the epinephrine. On this basis vanadium may possibly prove of use in internal hemorrhages occurring in these organs.

10. With moderate (intravenous) injections the general blood pressure usually returns approximately to its normal level (or even below) several minutes before the constriction in the abdominal organs disappears.

11. There is an increase in the peristaltic movements of the intestines. But the local application of vanadium to a loop of intestine does not cause a local anemia or a contraction of the bowel wall as occurs with barium. These two elements also differ widely in their actions on the heart.

12. Smooth muscle, except the vessels and alimentary canal, (and perhaps certain nonstriated muscular elements in the spleen and kidney) does not seem to be affected by the metal. In toxic doses the substance acts upon the kidneys and gastrointestinal canal in a manner similar to that of other irritating metallic bodies.

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STUDIES ON THE CONVULSIVE REFLEX PRODUCED BY STRYCHNINE: I. HABIT

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Habit depends fundamentally upon the functional development of certain physical structures within the nervous system over which impulses continually pass with invariable exactness. As an impulse passes over a certain path this tract becomes more susceptible to succeeding impulses, the result being an easier and more complete performance of the act called into play by that particular impulse. These paths of habit exist not only in the brain but also in the spinal cord where they are continually engaged in reflex activities.

The development of habit pathways, in the cord especially, depends upon the presence of external stimuli which elicit certain rather well defined acts. It may be assumed in this connection that anything which may produce muscular movement even though that substance be within the cord and in the nerve cells themselves may be considered as much an external stimulus as any of the habit producing events of everyday life.

Strychnine acting on nerve cells in the spinal cord, the exact localization of which has not been definitely proved, produces muscular contractions, tetanic in nature, and which represent an enormous expenditure of nervous energy; and certainly, if long continued, must leave a lasting influence on the paths of motor discharge. So the question arises, will the impulses of strychnine spasms long continued leave pathways over which succeeding impulses of strychnine spasms can more easily travel? In other words, will the time required for, or the ease of the pro-

duction of, tetanus with a definite and constant dose of strychnine be reduced with each succeeding exhibition of the drug?

For these experiments frogs were selected as the most practicable animals to use. Two dozen frogs of approximately uniform weight, 25 grams, were taken and placed in separate wire cages, one dozen in each. Each dozen received an initial injection of $\frac{1}{8}$ mg. each of strychnine nitrate on the same day. Injections were all made into the dorsal lymph sac, $\frac{1}{3}$ cc. of distilled water containing the dose of strychnine used. Enough solution was made up at one time to outlast the course of the experiments in order to avoid chance of error in changing solutions. The time was in no case taken until the animal had developed complete tetanus, and then the time record for each series of injections consisted of an average of the dozen, or, as experiment proceeded, of the surviving animals. When the number of surviving frogs was reduced to four and six respectively the experiment was discontinued since the reliability of the results would probably be impaired by individual variations. In this way our results, if affected at all, would be strengthened since it is very likely that the most susceptible frogs would be the first to die, leaving the more resistant to the last. The following table gives the history of the injections in detail.

Tabulated data on injections of first dozen

INJECTION OF OCTOBER 18	INJECTION OF OCTOBER 25	INJECTION OF NOVEMBER 1	INJECTION OF NOVEMBER 9	INJECTION OF NOVEMBER 16	INJECTION OF NOVEMBER 23
38	20 $\frac{1}{2}$	18	5	16	10
35	1	23	17	16	12
32	23	30	9	16	17
33	24	24	17	20	15
32 $\frac{1}{2}$	17	36	21		
34	22	34	21		
31	14 $\frac{1}{2}$	27			
30	19	28			
25	22	25			
19 $\frac{1}{2}$	18	23			
26	13	26			
27	17	17			

Figures refer to time of onset of spasms given in minutes.

Frogs are dead where no figures are given.

Tabulated data on injections of second dozen

INJECTION OF OCTOBER 18	INJECTION OF NOVEMBER 15	INJECTION OF NOVEMBER 22	INJECTION OF DECEMBER 6
31	16	16	13
34	22	14	14
14	20	19	7
39	23	13	8
30½	14	13	12
27	20	12	9
33	20		
23	25		
21	26		
22			
20			
18			

The intervals between the injections were adjusted to rule out any possible suggestion of cumulative action. The injection interval was never less than a week and in the second dozen the first interval was one month. To be doubly sure of complete elimination of each dose of strychnine before the succeeding dose was given control frogs were injected with the same amount and tissues analyzed for strychnine by standard methods before the succeeding injection. Frogs dying during the course of the experiments were also analyzed. Results were found negative in each case as determined by the sulphuric acid and potassium bichromate color test.

Summarizing the experimental results we deduce the following table which shows clearly the marked shortening in the time required for the production of spasms. The figures give the average time in minutes for each series.

Table of averages for first dozen with injection interval

	INTERVAL	NUMBER OF FROGS	AVERAGE TIME
	days		minutes
Initial injection of October 18.....		12	30½
Injection of October 25.....	7	12	19
Injection of November 1.....	7	12	26
Injection of November 9.....	8	6	15
Injection of November 16.....	7	4	17
Injection of November 23.....	7	4	13½

Table of averages for second dozen with injection interval

	INTERVAL	NUMBER OF FROGS	AVERAGE TIME
	<i>days</i>		<i>minutes</i>
Initial injection of October 18.....		12	26
Injection of November 15.....	28	9	20 $\frac{3}{4}$
Injection of November 22.....	7	6	14 $\frac{1}{2}$
Injection of December 6.....	14	6	10 $\frac{1}{2}$

In examining the above results we find that for the first dozen the time of the onset of spasms was shortened from 30 $\frac{1}{4}$ minutes to 13 $\frac{1}{2}$ minutes in a period of 36 days, the total number of injections being six. In the second table the data are more in accord with theory; this is due probably to longer injection intervals. Here the time was shortened from 26 to 10 $\frac{1}{2}$ minutes in a period of 49 days, the total number of injections being four. In addition to a shortening of the time of onset of spasms the duration of the spasms was prolonged after each injection. Recovery from spasm after the initial injection occurred in about 18 hours, and after later injections his time was increased to 24, 36, and 48 hours, and in some individual instances to 72 hours. Thus the time required for recovery was found to be directly proportional to the number of injections. The number of tetanic convulsions elicited by any one of these injections would seem to be numerically sufficient to develop a habit.

Recalling the hypothetical question with which we started we find that with succeeding injections the time required for spasms to appear was shortened, in other words, a convulsive habit was formed. This can readily be associated with work of other investigators of strychnine action in which they find in continued experiments an increased susceptibility not only in frogs but in some of the higher animals, rabbit and dog especially. The term habit as used in this paper does not mean habit in the sense of increased or decreased tolerance, but habit in its strictest psychological sense, meaning that with repetition of certain movements succeeding movements of the same nature become more easy and certain of execution; and with the idea that all movement is fundamentally a reflex. Results here are perfectly analogous to develop-

ment of habit spasms or ties in people of neurotic disposition. They are also to be considered analogous to the increase frequency of the convulsive seizures of epilepsy, especially of traumatic origin, for in this condition it can not easily be assumed that the exciting spicule of bone, or whatever the underlying cause may be, increases its irritation intensity as time goes on; but rather that its influences are more easily transmitted to the cortical motor cells, and after having once reached them the path is thereafter more easily traversible even though the first period of latency extends over years.

Since sensory impressions in the form of external stimuli are apparently necessary to produce strychnine convulsions it follows that the intermediate neurons passing from sensory to motor side of cord must be one of the connecting links over which impulses pass. Under normal conditions the motor response to a sensory impression is, at least to a certain extent, in direct ratio to the intensity of the sensory stimulation. Strychnine disturbs this balance so that a very slight stimulus will produce a maximal motor response. This motor response must pass along certain definite pathways, some of which are probably never used under normal conditions. The number of impulses passing over these pathways even after one injection are very many, and with each succeeding convulsion must be deepened, or in other terms, become more susceptible to, and more easily traversed by, the stimuli which pass over them. As this susceptibility to impulses is increased the stimulus necessary to elicit the reaction is reduced, or, the same stimulus produces a typical motor response more easily than at first: in short, *a habit is formed*.



STUDIES ON THE CONVULSIVE REFLEX PRODUCED BY STRYCHNINE. II. AS MODIFIED BY EPINEPHRINE

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The purpose of this research is to determine what effect epinephrine has on the convulsions produced by strychnine, and whether or not epinephrine may be used as an antagonist for strychnine after spasms have developed in the intact animal.

Falta and Jocovic,¹ from experiments on the exposed heart of a frog, assert that epinephrine is a powerful antidote to strychnine; the strychnine depressed heart being stimulated to renewed activity by local application of epinephrine. This being so, a point of much more importance is to determine if the two drugs modify each other as regards the action of strychnine on the spinal cord and brain since strychnine affects these centers much earlier and more energetically than the heart.

Exner² reports cases in which guinea pigs and rabbits after intraperitoneal injection of epinephrine tolerate much larger doses of strychnine. From preliminary observations we found that in the case of frogs the action of epinephrine hastened rather than deferred the onset of spasms. Later this was also found to be true for guinea pigs. Our results with rabbits were not so uniform. Thus our results with the doses given are diametrically opposed to the above quoted findings on tolerance to strychnine as produced by epinephrine. To attempt a uniform correlation of Falta and Jocovic's experiments on the exposed heart and

¹ Berl. Klin. Woch. October, 1909, xlv, p. 1929.

² Zeit. f. Heilkunde Abteilung f. Chirurgie, 1903; Bd. 24. s. 302; also Zeit. f. Exp. Pathologie und Pharmacologie, 1903, I, s. 313.

the direct variance of Exner's experiments on guinea pigs with our early findings the following work was carried out.

A. STRYCHNINE DEPRESSION OF HEART AS MODIFIED BY
EPINEPHRINE

This part of the work is based on the experiments of Falta and Jocovic. Besides corroborating their experimental results it demonstrates the antagonistic action of epinephrine and strychnine to each other on some tissues and contrasts these results with later experiments on the cord in which a synergistic action is found. A frog of average weight (about 30 grams) was pithed, and the heart exposed. The heart movements were transmitted to the drum by a small cork float resting on the ventricle of the heart and attached to a lever above by means of a perpendicular wire. In this arrangement the heart was not traumatized. Systole is indicated by the downward stroke in the tracing.

The following abbreviated protocol in connection with the tracings is self explanatory.

- 2:47 $\frac{1}{4}$ cc. of 2 per cent strychnine nitrate dropped directly on heart.
- 2:54 diastole beginning to lengthen.
- 3:02 marked irregularity.
- 3:06 $\frac{1}{4}$ cc. of 2 per cent strychnine nitrate on heart.
- 3:09 $\frac{1}{4}$ cc. of 1:1000 epinephrine solution.
- 3:11 marked acceleration followed by series of auricular contractions with few ventricular contractions.
- 3:16 Heart rhythm restored to normal and ventricular contractions increased in amplitude.

It will be noticed that there is an antagonism of epinephrine to strychnine but this does not differ from antagonism to other cardiac depressants for replacement of strychnine by chloral hydrate gave similar results. After the first application of epinephrine to the heart it required a larger amount of strychnine a longer time to produce slowing and irregularity, indicating that in these doses the stimulating effect of epinephrine was greater than the paralyzing action of strong solutions of strychnine.

B. LEG REFLEXES AS MODIFIED BY EPINEPHRINE

Before commencing the work showing the relation of epinephrine to strychnine on the cord it was thought advisable to determine just how much action epinephrine alone has on the central nervous system as determined by reflexes. This was obtained by dipping the legs, separately, of pithed frogs of uniform weight (25 grams) into a weak solution of acetic acid before and after injection of varying amounts of epinephrine as indicated in the tables. Reflexes were determined in one to two minute intervals before and after injection, allowing five minutes after injection for drug to begin to produce its effects.

Following table is illustrative of typical findings.

Normal reflexes

FROG 1		FROG 2		FROG 3		FROG 4	
Right leg	Left leg	Right leg	Left leg	Right leg	Left leg	Right leg	Left leg
<i>sec.</i>	<i>sec.</i>	<i>sec.</i>	<i>sec.</i>	<i>sec.</i>	<i>sec.</i>	<i>sec.</i>	<i>sec.</i>
3	3	3	2	2 $\frac{1}{4}$	4	4	4
3	3	2	2 $\frac{1}{2}$	2	3 $\frac{1}{2}$	5	4 $\frac{1}{2}$
3	2	2 $\frac{1}{2}$	2 $\frac{1}{4}$	2	2 $\frac{1}{2}$	4	3
3	1	3	2	2 $\frac{3}{4}$	2 $\frac{1}{2}$	4	3
3	3	3	2 $\frac{1}{2}$	2 $\frac{1}{2}$	1 $\frac{3}{4}$	3 $\frac{1}{2}$	3 $\frac{1}{2}$
		2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{1}{4}$	4	3

Reflexes after epinephrine

$\frac{1}{2}$ cc.		$\frac{1}{3}$ cc.		$\frac{1}{3}$ cc.		$\frac{1}{3}$ cc.	
1	1	3	3	3 $\frac{1}{2}$	3	4 $\frac{1}{2}$	4 $\frac{1}{2}$
1	1 $\frac{1}{2}$	4	3 $\frac{1}{2}$	5	4	5	5
1	2	2 $\frac{1}{2}$	2 $\frac{1}{2}$	3 $\frac{1}{2}$	4 $\frac{1}{4}$	4 $\frac{3}{4}$	5 $\frac{1}{2}$
1	1 $\frac{1}{2}$	2 $\frac{1}{2}$	2	6	6	5	5
1 $\frac{1}{2}$	1	3	2 $\frac{1}{2}$	5	7	6	6
1 $\frac{1}{2}$	1					6 $\frac{1}{2}$	7
						8	8

Out of this series and others not published frog 1 is the only instance found where the reflex time was shortened. Other cases showed no appreciable change, but in the majority of instances the reflex time was found to be lengthened and if taking of reflexes was

continued beyond the time shown in the table the movements became sluggish and later failed to appear entirely. This slowing of reflex time was more marked in larger doses as shown in frog 4 in above table. The experiments could not be prolonged to any marked degree because of a complicating fatigue factor vitiating results. This slowing of reflex response to acid is comparable to similar condition produced by injection of strychnine alone. The inference that epinephrine and strychnine act on similar or related structures is not untenable, hence, without binding proof, we may assume this to be so. The depressant action of epinephrine in larger doses becomes evident by examination of following table where the dose was doubled and the weight of the frogs decreased.

TIME OF TESTING REFLEXES	EPINEPHRINE FROG A		CONTROL FROG B		EPINEPHRINE FROG C	
	Right leg	Left leg	Right leg	Left leg	Right leg	Left leg
	sec.	sec.	sec.	sec.	sec.	sec.
2:59	4	5	3	2½	5	3
3:06	4	7	7	5	10	7
3:11	6	5	5	5	9	6
3:14	⅔ cc. epinephrine in abdominal lymph sac.				⅔ cc. epinephrine in abdominal lymph sac.	
3:18	No response in 30 sec- onds.		4	7	No response in 30 sec- onds.	
3:20	Heart beat feeble.		5	5	Heart beat feeble and ventricle contracted.	
3:25	No response in 60 sec- onds.		4	4	No response in 60 sec- onds.	

C. ACTION OF EPINEPHRINE IN RELATION TO STRYCHNINE SPASMS

Intact frogs were next injected with a mixture of epinephrine and strychnine; and with epinephrine before, and after, injection of strychnine with interesting results. The following table illustrates typical findings with injection of epinephrine and strychnine simultaneously and of strychnine after epinephrine.

FROG 1	EPINEPHRINE FOLLOWED IN EIGHT TO TEN MINUTES WITH STRYCHNINE	STRYCHNINE CONTROL
1	$\frac{1}{4}$ mg. * strych. and $\frac{1}{3}$ cc. epi. tet. in 1 min.	$\frac{1}{4}$ mg. strych. tet. in $5\frac{1}{2}$ min.
2	$\frac{1}{4}$ mg. * strych. and $\frac{1}{3}$ cc. epi. tet. in $3\frac{1}{2}$ min.	$\frac{1}{4}$ mg. strych. tet. in $5\frac{1}{2}$ min.
3	$\frac{1}{8}$ mg. * strych. and $\frac{1}{3}$ cc. epi. tet. in 1 min.	$\frac{1}{8}$ mg. strych. tet. in 20 min.
4	$\frac{1}{8}$ mg. strych. tet. in 8 min.	$\frac{1}{8}$ mg. strych. tet. in 17 min.
5	$\frac{1}{8}$ mg. strych. tet. in 9 min.	$\frac{1}{8}$ mg. strych. tet. in 16 min.
6	$\frac{1}{8}$ mg. strych. tet. in $12\frac{1}{2}$ min.	$\frac{1}{8}$ mg. strych. tet. in $14\frac{1}{2}$ min.
7	$\frac{1}{8}$ mg. strych. tet. in 10 min.	$\frac{1}{8}$ mg. strych. tet. in $30\frac{1}{4}$ min.†
8	$\frac{1}{8}$ mg. strych. tet. in 19 min.	$\frac{1}{8}$ mg. strych. tet. in $30\frac{1}{4}$ min.†
9	$\frac{1}{6}$ mg. strych. tet. in 16 min.	$\frac{1}{6}$ mg. strych. tet. in 27 min.

*Mixture; not followed by epinephrine alone.

†Time is average of 12 frogs, variation in individual time ranging from $19\frac{1}{2}$ to 38 minutes.

Frogs injected with epinephrine alone all showed a marked depression after 1 to 10 minutes depending on the dose given. A primary stimulation seemed to be present in some cases but with the doses given here it was not definite or constant enough to be of any great importance. In addition to depression in which the animal assumed a rather characteristic position with legs well drawn up to the body and the head bent down, there was also distinct evidence of increased glandular activity, the secretion of mucous and serous glands of skin and mucous membranes of nasal region being increased. Glandular structures in general were stimulated.

In the presence of such noticeable depression one would expect that strychnine action would be delayed. Quite the contrary, however, was observed, animals becoming tetanic in less time than the normal control frogs. In epinephrine frogs there was somewhat less irritability previous to convulsions than in control frogs, in other words, a somewhat greater stimulus was required to produce a response than under strychnine alone. Some frogs after injection of epinephrine and strychnine were removed to a quiet place to minimize external stimuli as much as possible to see if epinephrine itself would safeguard frogs from convulsions, but spasms appeared in about the same length of time as in the other frogs under the usual experimental conditions.

Would large doses of epinephrine more readily influence spasms?

Three frogs were given $\frac{1}{6}$ mg. of strychnine each, tetanus following in $5\frac{1}{2}$, 8, and 7 minutes. Two of these frogs after the onset of spasms were given 1 cc. doses of 1:1000 epinephrine but no difference in intensity of spasms from those of control frog could be detected. Later one of these two frogs was given an additional 1 cc. but without effect. Three other frogs were each given 1 cc. of epinephrine which produced marked depression in a few minutes. These were then given $\frac{1}{6}$ mg. of strychnine each, tetanus following in 5, 6 and $4\frac{1}{2}$ minutes. Here, again, in spite of large doses, epinephrine fails to exert any beneficial effect after spasms have been produced and if injected previous to strychnine the spasms come on more quickly.

Epinephrine injected after the onset of strychnine spasms has no appreciable effect on convulsions, and if injected after smaller doses of strychnine and before onset of spasms allowing 5 to 10 minutes to elapse between the two injections the time of onset of spasms is not hastened or delayed as determined by control experiments, due to the fact that strychnine action has progressed too far for epinephrine to be a factor.

These injections were repeated on guinea pigs, injections being subcutaneous, the results paralleling those on frogs. Following table gives data.

WEIGHT OF PIG	INJECTIONS GIVEN	TIME REQUIRED TO PRODUCE SPASMS
<i>grams</i>		<i>minutes</i>
230	$\frac{1}{2}$ cc. 1:1000 epinephrine followed in $5\frac{1}{2}$ minutes by 3 mg. of strychnine.....	$1\frac{1}{2}$
230	3 mg. of strychnine.....	5
242	Mixture of $\frac{1}{4}$ c.c. 1:1000 epinephrine and $1\frac{1}{2}$ mg. of strychnine.....	3
240	$1\frac{1}{2}$ mg. of strychnine.....	13
245	Mixture of $\frac{1}{4}$ cc. 1:1000 epinephrine and $1\frac{1}{4}$ mg. of strychnine.....	5
195	$1\frac{1}{4}$ mg. of strychnine.....	12

Here also, the results are perfectly clear, and injection of additional doses of epinephrine immediately before or after the onset of spasms was not sufficient even in large doses to avert spasms or death.

In repeating Exner's experiments on rabbits, giving strychnine by stomach, we could not get so uniform results as those reported by him. The following table includes the results both of this repetition and of cases where strychnine was given subcutaneously.

WEIGHT OF RABBITS	TIME AND NATURE OF INJECTIONS	TIME REQUIRED TO PRODUCE	
		Spasms	Death
<i>grams</i>		<i>minutes</i>	<i>minutes</i>
870	2:20 4 mg. strychnine (stomach)		
	2:38 Convulsion	18	
	2:39 Dead		19
1100	2:18 4 mg. strychnine (stomach)		
	2:48 Marked irritability		
	3:00 Spasms on stimulation. Animal fully re- covered on following morning	42	
820	2:05 $\frac{1}{2}$ cc. epinephrine* (intraperitoneally)		
	2:24 $\frac{1}{2}$ cc. epinephrine (intraperitoneally)		
	2:30 4 mg. strychnine (stomach)		
	2:55 Slight irritability		
	3:51 Spasms on stimulation		
800	3:55 Tetanus	85	
	4:40 Dead		130
	2:06 $\frac{1}{2}$ cc. epinephrine (intraperitoneally)		
	2:27 4 mg. strychnine (stomach)		
	2:50 Convulsion	23	
1100	2:51 Dead		24
	3:32 1 cc. epinephrine (intraperitoneally)		
	3:44 4 mg. strychnine (subcutaneous)		
	3:51 Convulsion	7	
	3:58 Dead		14
970	10:15 1 cc. epinephrine (subcutaneous)		
	10:46 1 mg. strychnine (subcutaneous) No well defined spasms appeared.		
820	10:47 1 mg. strychnine (subcutaneous)		
	11:01 Convulsion	14	
	11:06 Dead		19
1870	11:26 1 cc. epinephrine (subcutaneous)		
	11:47 2 mg. strychnine (subcutaneous). No spasm appeared.		
1200	11:17 1 cc. epinephrine (subcutaneous)		
	11:23 $1\frac{1}{2}$ mg. strychnine (subcutaneous)		
	11:38 Convulsions	15	
	11:41 Dead		18

* In these experiments Solution of Adrenalin Chloride (Parke, Davis and Company) was used. Throughout remainder of experiments Supracapsulin (Cudahy Packing Company) was used.

In some animals the presence of epinephrine had no effect in delaying spasms, and in others some delay was found. This variation in the time of onset seems to be influenced by the time interval elapsing between the two injections, that is, the greater the time permitted to elapse after the epinephrine and before the strychnine injection the longer will it take for convulsions to appear. This accounts for the variance of results between guinea pigs and rabbits, since, in the guinea pig series only $5\frac{1}{2}$ minutes elapsed, while in the rabbit series the time interval ranged from 6 to 31 minutes between the two injections. One rabbit, where strychnine was given 6 minutes after epinephrine, died in 18 minutes, while control rabbit, without epinephrine, died in 19 minutes. In others where a longer time interval elapsed the onset of spasms was decidedly delayed, although in one rabbit (800 grams) the time of appearance of convulsions and death did not differ markedly from the control rabbit (870 grams).

DISCUSSION

The salient points of above work on strychnine action as modified by epinephrine divide themselves into three groups, (1) antagonistic action on the heart, (2) an apparent antagonism on the cord, and (3) a synergistic action on the cord. Thus on the one hand epinephrine may be considered antagonistic to strychnine and on the other synergistic depending on the point of action. In the light of the results recorded above, Falta and Jocovic's assertion that epinephrine is a powerful antidote to strychnine requires important modifying statements, and from the standpoint of practical application it is incorrect.

In interpretation of results the findings on the exposed heart need not detain us long. The action of strychnine here was local, strong solutions acting as a protoplasmic poison on muscle cells themselves, and on the local nervous mechanism of the heart; the result being depression of the heart even to the point of stopping it in diastole. Epinephrine, being stimulant to muscle fibers and to sympathetic accelerators, restored the depressed heart to an activity even greater than normal. In this

state the heart could tolerate larger doses of strychnine thus showing that epinephrine applied locally to the heart is a real antagonistic drug to strychnine.

Regarding the apparent antagonism on the cord we accept the explanation given by Exner, namely, that it is mainly a question of delayed absorption. In addition we believe that shock and metabolic changes, including some of the products formed, carbon dioxide and sugar especially, may play some part in inhibiting the spasm. When a longer time is allowed to intervene between the injection of the epinephrine and strychnine more uniform results are to be expected because more time is given for the symptoms of shock to develop and also for the accumulation of waste products. A detailed investigation of these assumptions is not warranted by the questions concerned. In no case is there any indication of a specific antagonism as claimed by Falta and Jocovic.

On the cord, however, epinephrine in its final results showed quite a different behavior; the drug instead of inhibiting the spasm actually hastened them. The interpretation of the nature of the mechanism involved in the synergistic action shown in relation to strychnine on the cord is more complicated.

The action of epinephrine on the brain is first a stimulation followed rapidly by a paralytic action which lessens cerebral inhibition much the same as pithing. The stimulating action may be so transient that with larger doses it is not apparent. The action on the cord appears to be the same as on the brain, but the stimulating action is more prolonged and paralysis, at least of some elements, comes on much later. This would account for the intensified strychnine action after epinephrine.

It seems to us, however, most probable that epinephrine first stimulates and then depresses the structures upon which convulsions depend, and that in convulsions produced by strychnine alone there is a paralytic factor involved. We know from experiments in which epinephrine is given simultaneously with strychnine that the spasms appear with the same shortened time interval as when epinephrine is given 8 to 10 minutes previous to strychnine. From this it must follow that the depression produced by epinephrine must occur much more quickly than the

depression required for spasms under strychnine alone. Therefore, in the presence of epinephrine this paralysis takes place sooner and strychnine can in consequence act more easily. So far as the cord alone is concerned we must conclude that the two drugs are synergistic in action.

SUMMARY

1. Epinephrine is antagonistic to the paralytic action of strong strychnine solutions on the heart.
2. Epinephrine and strychnine have a synergistic action on the cord. Spasms develop more quickly when epinephrine is given with or previous to strychnine.
3. Strychnine is antagonistic to the general depression produced by epinephrine, but this antagonism is not mutual. Epinephrine will not antagonize a strychnine spasm.
4. From the above findings there is no indication that epinephrine can be applied with benefit in the treatment of strychnine convulsions.

ON THE CONVULSANT ACTION OF SOME SULPHONATED DYES

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Barbour and Abel, in a preceding issue of this journal,¹ have called attention to the striking action of acid fuchsin on frogs. They have shown that this substance injected into frogs in doses of 1 to 4 mgs. per gram of body weight will produce flexor spasms and convulsions, appearing in from one to twenty hours after injection, which in their turn give way to an extensor tetanus. They have further pointed out a most interesting fact, that these convulsions and tetanus can be produced much more quickly (in from one-half to thirteen minutes) and by much smaller quantities of the drug (as little as 0.35 mg.² per gram of body weight) if the anterior third of the cerebrum be cut off from the rest of the central nervous system.

It was interesting to learn whether the above pharmacological properties were peculiar to acid fuchsin only, or were to be found in other drugs. Accordingly a search for other substances with similar properties was made by the author in this laboratory to determine this point.

A large number of drugs were studied, among them various phenols (phenol, pyrogallol, resorcin), pikrotoxin, minute doses of strychnine, atropin, and a long list of dyes: carmine red, methylene blue, various Congo reds, methyl orange, methyl violet, several varieties of scarlet red, tropaeolin, naphthol yellow, phenol-sulphone-phthalein, etc. Many of these substances were found to produce convulsions and tetanus, in some only when in-

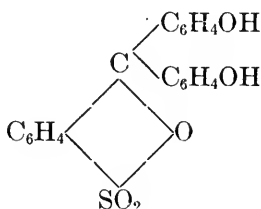
¹ Jour. of Pharm. and Exper. Therap., vol. ii, No. 3, December, 1910, p. 167.

² Later experiments made by Professor Abel and myself seem to point to a still smaller minimal dose—0.2 mg. per grams weight.

jected in minute quantities and appearing very late, as for instance in the case of atropin, a fact to which attention was called long ago by Fraser,³ but which seems to be very little known. None of them showed, however, any difference in their action in respect to the removal of the anterior third of the cerebrum, with the exception of the dyes—*phenol-sulphone-phthalein*, *naphthol yellow*, and *tropaeolin* and *Basel I* and *Basel III*. These bodies were found to act precisely in the same way as acid fuchsin, as will be seen from the experiments which follow.

THE ACTION OF PHENOL-SULPHONE-PHTHALEIN

Phenol-sulphone-phthalein is one of a class of compounds analogous to the phthaleins and was first prepared by Remsen⁴ and further described by his pupil, Sohon.⁵ It has recently, through the researches of Abel and Rowntree,⁶ and then of Rowntree and Geraghty,⁷ gained great importance from its use in functional tests for renal activity. The structure of this interesting substance is represented by the formula



It is a bright red crystalline powder, soluble in water, more so in alcohol, and insoluble in ether. Its dilute alkaline solution is of purer red than that of phenol-phthalein, while a more strongly alkaline solution is purple. A 5 per cent solution of the sodium salt was used in our experiments. The sodium salt solutions are prepared as follows: One gram of phenol-sulphone-phthalein and 1.4 cc. of 2 N sodium hydroxide are added to 18.6 cc. of either $\frac{N}{23}$

³ Fraser, Edinburgh Royal Society, vol. xxv, p. 449, 1868.

⁴ Amer. Chem. Jour., vi, 180.

⁵ Amer. Chem. Jour., xx, 257.

⁶ Jour. of Pharm. and Exper. Therap., vol. i, no. 2, p. 231.

⁷ Ibid., p. 579.

sodium chloride solution or distilled water. This gives a solution of the mono-sodium or acid salt, which is red in color and which if injected in this form produces slight local irritation. It is therefore necessary to add from five to ten drops more of 2 N sodium hydroxide solution, a quantity of alkali which is sufficient to change the color to a beautiful Bordeaux red, and entirely to counteract the irritating effect of the acid salt but insufficient for the purplish red neutral salt.

Large quantities of the 5 per cent solution when injected into the lymph sac of a frog were found to have either no effect at all, or to produce convulsions in an hour or longer after injection. On the other hand when the anterior third of the cerebrum was removed, much smaller quantities of the drug (0.52 mg. to 1.62 mg.) produced violent convulsions followed by tetanus in a very short time—one half to thirty-five minutes, as will be seen from the following procotols and table.

Experiments illustrating the action of phenol-sulphone-phthalein

Experiment No. 1. December 1, 1910. *Rana pipiens*. Weight, 20 grams.

- 3.45 p.m. Injected intraperitoneally, 10 minims = 1.56 mg. per gram of frog of 5 per cent solution of phenol-sulphone-phthalein.
- 4.00 p.m. Frog is lively and in normal condition.
- 4.02 p.m. Ablation of the anterior one-third of the brain. There is very little bleeding, and very little depression.
- 4.08 p.m. Frog is more excitable; jumps and quivers at the slightest touch.
- 4.10 p.m. Spontaneous clonic convulsions and tetanus.
- 4.20 p.m. Dead. Examination of the tissues shows the brain and spinal cord deeply stained by the dye.

Control Experiment No. 1. Exactly the same quantity per gram of frog was given, but no operation performed. No convulsions were observed that afternoon.

Experiment No. 5. December 9, 1910. *Rana pipiens*. Weight 30 grams.

- 12.25 p.m. Injected 7 minims = 0.73 mg. per gram of frog in abdominal lymph sac, of 5 per cent solution of phenol-sulphone-phthalein.
- 12.33 p.m. Lively and active.

12.37 p.m. Ablation of anterior one-third of brain.

12.38 p.m. Immediate rigidity and extensor tetanus, belly distended.

12.40 p.m. Momentary relaxation followed by tetanus again.

Control Experiment No. 5. Exactly the same quantity per gram of frog of the drug was injected, but no operation performed. No convulsions were observed that afternoon.

Experiment No. 6. December 2, 1910. *Rana pipiens*. Weight, 19 grams.

12.25 p.m. Injected intraperitoneally 10 minims of 5 per cent solution phenol-sulphone-phthalein = 1.64mg. per gram frog.

12.45 p.m. Normal.

2.00 p.m. Some excitability.

4.00 p.m. Strong convulsions, but no tetanus noted.

5.00 p.m. Death. Autopsy shows large quantities of the pigment everywhere, especially in brain and spinal cord.

Experiment No. 7. December 5, 1910. *Rana pipiens*. Weight, 25 grams.

4.13 p.m. Injected in dorsal lymph sac 5 minims of 5 per cent solution phenol-sulphone-phthalein = 0.63mg. per gram frog.

4.23 p.m. Anterior third of the brain cut off. Immediate depression following operation and lasting one minute.

4.28 p.m. Exceedingly sensitive to touch.

4.37 p.m. Hyperexcitable.

4.45 p.m. Laid on back, cannot resume natural position, and shows suggestion of convulsions.

4.50 p.m. Tetanic convulsions; extremities extended.

4.52 p.m. Continued convulsions.

4.55 p.m. Tetanus and opisthotonus.

4.57 p.m. Stiff as in strychnine poisoning with arms clasped and legs stretched out and held close together.

Control Experiment No. 7. Exactly same dose of drug per gram frog was given, but no operation performed. No tetanus or convulsions were observed.

Experiment No. 12. December 13, 1910. *Rana pipiens*. Weight, 38 grams.

December 8. A bloodless operation was performed, dividing the anterior one-third of the brain from the rest of the central nervous system.

December 13 Wound perfectly healed. Frog behaves like a normal animal.

- 11.00 a.m. Injected in dorsal lymph sac 8 minims of 5 per cent solution of phenol-sulphone-phthalein = 0.65 mg. per gram of frog.
11.05 a.m. Hyperexcitability noted.
11.08 a.m. Tetanic convulsions.
11.30 a.m. Frog lies "dead" with constant quivering of muscles.

Control Experiment No. 12. A bloodless operation similar to the one in the preceding experiment was performed, but no drug was later injected. *No convulsions were ever observed*, the animal behaving as if it were normal.

Experiment No. 16. March 9, 1911. *Rana clamata*. Weight, 31 grams.

- 1.10 p.m. Injected in dorsal lymph sac 6 minims of 5 per cent solution of phenol-sulphone-phthalein = 0.58 mg. per gram frog.
1.35 p.m. Ablation of anterior third of brain. Slight depression following operation.
1.40 p.m. Recovered—normal.
2.00 p.m. Hyperexcitability noted.
2.23 p.m. Rigidity of legs.
2.33 p.m. Tetanic convulsions.
2.40 p.m. Beautiful tetanus with extremities extended.
2.45 p.m. Tetanus continues.

Control Experiment No. 16.

- 1.08 p.m. To a *Rana clamata* weighing 30 grams the same dose of phenol-sulphone-phthalein = 0.58 mg. per gram frog was given in dorsal lymph sac, but no operation was performed.
1.35 p.m. No change.
2.00 p.m. No change.
3.00 p.m. No change.

Experiment No. 18. March 9, 1911. *Rana clamata*. Weight, 32 grams.

- 2.40 p.m. Anterior third of the brain is divided from the rest of the central nervous system, with very little bleeding. No post-operative or other depression.
2.45 p.m. Injected 15 minims of 5 per cent solution of phenol-sulphone-phthalein = 1.46 mg. per gram of frog.
2.50 p.m. Hyperexcitable.
2.55 p.m. On touching rigidity is noticed.
3.00 p.m. Tetanus.

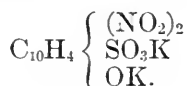
Control Experiment No. 18. On dividing anterior third of brain, without subsequent injection of drug, no convulsions occur.

TABLE I
Sulphone-phthalatein

NUMBER OF EXPERIMENT	RANA SPECIES	WEIGHT grams	DOSE PER 1 GRAM OF BODY WEIGHT	PLACE OF INJECTION	TIME FROM INJECTION TO OPERATION minutes	OPERATION	TIME FROM OPERATION TO SYMPTOMS hrs. min.	SYMPTOMS	
								Premontory	Tetanus
1	Piptens	20	1.56	Intraperitoneal	17	Anterior third cerebrum	8	Primary excitability	Convulsions and tetanus.
2	Piptens	20	1.56	Dorsal lymph sac	30	Anterior third cerebrum	12	Primary excitability	Violent convulsions, tetanus, and opisthotonus
3	Piptens	36	0.26	Abdominal lymph sac	27	Anterior third cerebrum	30	Slightly hypersensitive	No convulsions same day and next day
4	Piptens	28	1.12	Abdominal sac	23	Anterior third cerebrum	7	Primary excitability	Convulsions and tetanus
5	Piptens	30	0.73	Abdominal sac	12	Anterior third cerebrum	1½		Immediate extensor tetanus
6	Piptens	19	1.64	Intraperitoneal		None	3	Convulsions	
7	Piptens	25	0.63	Dorsal sac	10	Anterior third cerebrum	22	Convulsions	Tetanus
8	Piptens	21	0.74	Dorsallymph, sac		None	1 20	Convulsions	Tetanus
9	Piptens	18	0.86	Intraperitoneal		None	1 30	No symptoms	Death on following day
10	Piptens	30	0.52	Intraperitoneal	33	Anterior third cerebrum	25	Convulsions	Limbs flexed
11	Piptens	35	0.31	Dorsal sac	20	Anterior third cerebrum	1 40	Primary depression no convulsions	Death on following day
12	Piptens	38	0.65	Dorsal sac		Anterior third cerebrum	8	Convulsions	Bloodless operation 5 days previously
13	Piptens	12	0.65	Abdominal sac	18	Anterior third cerebrum	2	Convulsions	
14	Piptens	17	0.64	Abdominal sac	38	Anterior third cerebrum	35	Convulsions	Tetanus and opisthotonus
15	Clamata	35	0.80	Dorsal sac	23	Anterior third cerebrum	35	Primary depression	Tetanus and opisthotonus
16	Clamata	32	0.58	Dorsal sac	25	Anterior third cerebrum	25	Hyperexcitability	Tetanic convulsions
17	Clamata	30	1.56	Dorsal sac	5	Anterior third cerebrum	5	Convulsions	Tetanus
18	Clamata	32	1.46	Abdominal sac		Anterior third cerebrum	5	Hyperexcitability	Legs abducted
19	Piptens	20	1.56	Dorsal lymph sac		Anterior third cerebrum	12		Previously operated

THE ACTION OF NAPHTHOL YELLOW

Naphthol yellow, or Naphthol Gelb S, is a brilliant yellow dye discovered by H. Caro in 1879 and further studied by Paul Lauterbach.⁸ It is the potassium salt of di-nitro-naphthol-sulphuric acid, and has the following structural formula.



The salt consists of lemon yellow crystals difficultly soluble in hot water. On heating it turns red. A 5 per cent solution of this dye was used in our experiments. Injections of 2 mgs. or more per gram of body weight of this substance into an intact frog will produce either no effect except some depression or late convulsions usually appearing over an hour after injection. In decerebrate frogs, however, convulsions and tetanus are produced in a very short time 1 to 28 minutes after injection, and by a much smaller quantity of the drug, 0.57 to 1.44 mg. per gram weight of frog. The following protocols and table will illustrate this action.

Experiment No. 1. February 7, 1911. *Rana clamata*. Weight 30 grams.

- 1.18 p.m. Injected in dorsal lymph sac 6 minims of 5 per cent solution of naphthol yellow = 0.62 mg. per gram frog.
- 1.30 p.m. Anterior third of cerebrum cut. Immediate depression for one minute followed by recovery.
- 1.36 p.m. Spontaneous convulsions of short duration.
- 1.39 p.m. Spontaneous tetanic convulsions; opisthotonus; legs extended, and slightly abducted; tetanus lasts for a minute at a time; there is also a slight tremor of the body; respirations rapid.
- 2.15 p.m. Practically dead.

Control Experiment No. 1. *Rana clamata*. Weight 30 grams.

- 12.31 p.m. February 7. Injected 7 minims of 5 per cent solution of naphthol yellow in dorsal sac = 0.62 mg. per gram frog.
- 2.19 p.m. No change.
- February 9. Alive and well.

⁸ P. Lauterbach, Ber. der Deutch. Chem. Gesel. xiv, p. 2028-2029, 1881.

Experiment No. 11. January 13, 1911. *Rana pipiens*. Weight, 80 grams.

12.30 p.m. Bloodless operation performed dividing anterior third of cerebrum from the rest of the central nervous system.

January 17 Frog is alive and well.

11.05 a.m. Injected 25 minims of 5 per cent solution of naphthol yellow in dorsal and abdominal lymph sacs; no depression followed.

11.07 a.m. Spontaneous spasms or jerks lasting a second at a time. Veratrine-like action; frog stretches legs out but cannot pull them back; when placed on back, cannot turn over.

11.48 a.m. Spontaneous tetanus and opisthotonus.

Control Experiment No. 11. *Rana pipiens*. Weight, 23 grams.

3.37 p.m. January 9. Injection of 6 minims of 5 per cent solution naphthol yellow in dorsal sacs = 0.82 mg. per gram frog. Frog lively.

January 11. Alive and well.

January 12. Alive and well.

January 13. Alive and well.

January 17. Alive and well.

January 23. Alive and well.

Experiment No. 5. January 9, 1911. *Rana pipiens*. Weight, 24 grams.

2.43 p.m. Injected in dorsal lymph sac 6 minims of 5 per cent solution of naphthol yellow = 0.78 mg. per gram frog.

2.35 p.m. Anterior one-third of cerebrum cut off; immediate effect is slight depression; followed by recovery.

2.56 p.m. On touching, frog has spasms or convulsions.

2.58 p.m. A momentary tetanus.

2.59 p.m. Extensive tetanus and opisthotonus lasting a few seconds.

3.02 p.m. Spontaneous tetanic convulsions and opisthotonus.

3.12 p.m. Same.

3.15 p.m. Tetanus with bending of body sideways; twitching of toes.

Control Experiment No. 5. A corresponding dose of drugs per gram frog was injected, but no operation performed. No convulsions were observed.

Experiment No. 6. January 31, 1911. Effect of excessive dose. *Rana clamata*. Weight, 30 grams.

12.15 p.m. Injected 12 minims of 5 per cent solution naphthol yellow = 1.25 mg. per gram frog.

- 12.25 p.m. Anterior one-third of cerebrum is cut off. Immediate effect is great depression; frog is severely poisoned and does not recover. Death in a few minutes.

Experiment No. 10. January 2, 1911. An exceptional control frog. *Rana pipiens*. Weight, 16 grams.

- 11.16 a.m. Inject 8 minims of 5 per cent solution naphthol yellow in abdominal sac = 1.56 mg. per gram frog.

- 11.45 a.m. Spontaneous convulsions with extensor tetanus; arms clasped; trunk bent sideways.

Experiment No. 12. January 2, 1911. *Rana pipiens*. Weight, 26 grams.

- 11.15 a.m. Injected 12 minims of 5 per cent solution naphthol yellow in abdominal lymph sac = 1.44 mg. per gram frog.

- 11.25 a.m. Cut off anterior one-third of brain. Immediate depression, but recovers soon.

- 11.26 a.m. Tetanic convulsions, legs extended; fibrillary tremor of toes.

- 11.50 a.m. Tetanus repeated.

- 11.35 a.m. Dead. On autopsy brain and cord are found to be stained yellow.

Control Experiment No. 12. To a frog a proportional dose of drug was given, but no operation performed: no convulsions occurred.

Experiment No. 15. February 9, 1911. *Rana clamata*. Weight, 19 grams. Previously bloodlessly operated, so as to separate anterior one-third of cerebrum.

- 2.47 p.m. Inject 5 minims = 0.72 mg. per gram frog of 5 per cent solution of naphthol yellow in dorsal lymph sac.

- 2.50 p.m. Depressed and toxic; movements sluggish.

- 2.52 p.m. Placed on its back animal cannot turn over.

- 2.56 p.m. Sudden tetanus, with legs extended.

- 3.00 p.m. Tetanus with legs and arms tightly flexed and adducted.

Control Experiment No. 15. Injection of same dose of drug in intact frog produces no convulsions. Operated frog without injection of drug has no convulsions.

TABLE II
Naphthol yellow

NUMBER OF EXPERIMENT	RANA SPECIES	WEIGHT	DOSE PER GRAM OF FROG	PLACE OF INJECTION	TIME FROM INJECTION TO OPERATION	OPERATION	TIME FROM OPERATION TO SYMPTOMS	SYMPTOMS		Remarks
								Premontory	Tetanus	
		<i>grams</i>			<i>minutes</i>		<i>minutes</i>			
1	Clamata	30	0.62	Dorsal	12	Anterior third cerebrum	6	Immediate depression followed by	Convulsions, tetanus, opisthotonus and death	Operated the day before Death
2	Piptens	80	0.97	Dorsal and ab- dominal		Anterior third cerebrum	2	Extension and	Tetanus	
3	Piptens	18	0.60	Dorsal sac	6	Anterior third cerebrum	5	Depression	Tetanus opisthot- onus and	
4	Piptens	27	0.57	Dorsal sac	10	Anterior third cerebrum	8		Tetanus	"Tired out" very soon Death
5	Piptens	24	0.78	Dorsal sac	12	Anterior third cerebrum	1	Depression followed by hyperexcitability	Tetanus	
6	Clamata	30	1.25		10	Anterior third cerebrum	0	Great depression	Opisthotonus	Very much poisoned
7	Piptens	28	0.78	Abdominal sac	10	Anterior third cerebrum	1	Almost immediate te- tanus first extensor then flexor		
8	Piptens	65	0.62 in two doses	Abdominal sac	20	Anterior third cerebrum	7	First injection insuf- ficient	Tetanus and	Death
9	Piptens	20	0.64	Abdominal sac	20	Anterior third cerebrum	1		Tetanus	
10	Piptens	16	1.56	Dorsal sac		None	30		Convulsions	An exceptional con- trol frog
11	Piptens	15	0.62	Abdominal sac	10	Anterior third cerebrum			No convulsions	
12	Piptens	26	1.44	Abdominal sac	10	Anterior third cerebrum	1	Depression two to three minutes	Followed by tetanic convulsions	Death
13	Clamata	31	0.50	Dorsal sac		Anterior third cerebrum	5	Slight depression	None	
14	Piptens	26	0.84	Dorsal sac	35	Anterior third cerebrum	2	None	Tetanus, opisthotonus	Previously operated Subminimal dose Recovery
15	Clamata	19	0.72	Dorsal sac		Anterior third cerebrum	9	Depression followed by	Tetanic convulsions	
16	Clamata	46	0.81	Dorsal sac		Anterior third cerebrum	28	None	Tetanic convulsions	Previously operated
17	Clamata	38	0.82	Dorsal sac		Anterior third cerebrum	12	None	Tetanic convulsions	

THE ACTION OF TROPAEOLIN

The dye employed by us was the one called tropaeolin 00 or Orange IV. It belongs to the class of Azo Dye-stuffs⁹ and is the potassium salt of phenyl-amino azo-sulphuric acid.¹⁰ Its structural formula is



This substance consists of golden yellow needles, very difficultly soluble in cold, and more soluble in hot water. On heating it gives Indulin $\text{C}_{18} \text{H}_{15} \text{N}_3$. Tropaeolin is used as a dye and has also been used in gastric analysis for determining the presence of free HCl .¹¹ In the presence of free hydrochloric acid the yellow solution turns reddish yellow in color. This substance is more toxic than either phenol-sulphone-phthalein or naphthol yellow, so that after first using a 5 per cent solution, we used a weaker, or $2\frac{1}{2}$ per cent solution in later experiments.

On injection of Tropaeolin 00 into decerebrate frogs even very small quantities 0.15 mg. to 1.68 mg. per gram of body weight, cause convulsions and tetanus in from two to forty-five minutes. The same doses and even much larger doses injected into an intact frog, produce, with the exception of a depression, no convulsions at all, or the convulsions come on much later and are not so marked. The following protocols and table will illustrate the action of the drug.

Experiments illustrating the action of tropaeolin 00.

Experiment No. 1. December 22, 1910. *Rana pipiens*. Weight, 30 grams.

- 4.05 p.m. Injected in lymph sac 3 minims of 5 per cent. solution of tropaeolin = 0.31 gm. per gram frog.
- 4.15 p.m. Ablation of anterior third of cerebrum.
- 4.30 p.m. Slight depression.
- 4.41 p.m. Extensor tetanus and opisthotonus lasting one or two seconds.

⁹ Schmidt: Pharmaceutische Chemie, 1901, ii, 2, 1149.

¹⁰ Beilstein: vol. iv, p. 1370. (third ed.)

¹¹ Boas: Deutsche Med. Wehseft. 1877. vol. xiii. p. 852.

- 4.48 p.m. Tetanus of longer duration.
4.55 p.m. Tetanus with legs in Z-shaped position.
5.00 p.m. Still tetanic.

Control Experiment No. 1. To a *Rana pipiens* an equivalent quantity of tropaeolin was given, but brain left intact. No convulsions were observed.

Experiment No. 4. December 21, 1911. *Rana pipiens*. Weight, 22 grams.

- 11.05 a.m. Injected 2 minims of 5 per cent tropaeolin solution = 0.30 mg. per gram frog, in lymph sac.
11.20 a.m. Division of anterior third of brain.
11.30 a.m. Beginning rigidity.
11.39 a.m. Tetanus and opisthotonus.
2.30 p.m. Extensor tetanus.

Control Experiment No. 4. To a *Rana pipiens* an equivalent quantity = 0.30 mg. per gram frog was given, but no operation performed. No convulsions occurred.

Experiment No. 9. *Rana pipiens*. Weight, 16 grams.

- 4.20 p.m. Injected 3 minims of 5 per cent tropaeolin solution in abdominal sac. Immediate depression = 0.58 mg. per gram frog.
4.30 p.m. Anterior one-third of cerebrum cut off. No depression.
4.40 p.m. Placed on back, cannot turn over. Slight convulsions.
4.50 p.m. Very toxic.

Control Experiment No. 9. An equivalent amount of tropaeolin injected into an intact frog produced no convulsions.

Experiment No. 11. December 14, 1910. *Rana pipiens*. Weight, 18 grams.

- 12.10 p.m. Injected in abdominal lymph sac 10 minims of 5 per cent. tropaeolin solution = 1.62 mg. per gram frog.
12.15 p.m. Anterior third of cerebrum cut off. Immediate depression.
12.30 p.m. Recovered; responds to stimuli.
12.45 p.m. Veratrine like action.
12.45 p.m. Veratrine like action; slight convulsions.
1.45 p.m. Complete tetanus.

Control Experiment No. 11. An equivalent quantity of tropaeolin 1.52 mg. per gram frog injected in an intact animal produces no convulsions.

Experiment No. 13. December 13, 1910. *Rana pipiens*. Weight, 38 grams.

December 8, 1910. Bloodless operation performed, separating anterior third of cerebrum from the rest of the central nervous system.

11.00 a.m. December 13, 1910. Injected in dorsal lymph sac, 8 minims of 5 per cent. tropaeolin solution = 0.68 mg. per gram frog.

11.05 a.m. Hyperexcitability.

11.08 a.m. Tetanus.

11.30 a.m. Lies dead with muscles in fibrillation.

Control Experiment No. 13. An equivalent amount of tropaeolin injected into an intact frog produces no convulsions.

Experiment No. 20 February 23, 1911. *Rana clamata*. Weight, 38 grams.

1.05 p.m. Injected 5 minims of $2\frac{1}{2}$ per cent tropaeolin solution = 0.20 mg. per gram frog into dorsal lymph sac.

1.18 p.m. Cut off anterior third of cerebrum; very little bleeding. Postoperative depression lasting one minute.

2.03 p.m. Sudden spontaneous convulsions and extensor tetanus.

2.15 p.m. Marked flexor tetanus.

2.24 p.m. Tetanus with legs extended and opisthotonus.

Control Experiment No. 20. An equivalent dose of the drug injected into the lymph sac of an intact frog produced no convulsions.

Experiment No. 23. March 8, 1911. *Rana clamata*. Weight, 32 grams.

1.12 p.m. Injected 6 minims of $2\frac{1}{2}$ per cent tropaeolin solution = 0.29 mg. per gram frog into the dorsal lymph sac.

1.17 p.m. Cut off anterior one-third of cerebrum. Initial depression.

1.50 p.m. Prolonged and spontaneous tetanus and opisthotonus, constantly repeating itself for one and a half hours.

4.00 p.m. Dead.

Control Experiment No. 23. An equivalent dose of tropaeolin injected into the lymph sac of an intact frog produces no convulsions.

Experiment No. 25. March 8, 1911. *Rana clamata*. Weight, 28 grams.

2.25 p.m. Anterior third of cerebrum cut off, with very little bleeding and no depression following.

2.30 p.m. Injected 5 minims of a $2\frac{1}{2}$ per cent solution of tropaeolin = 0.28 gm. per gram frog in abdominal lymph sac.

3.12 p.m. Extensor and flexor convulsions.

3.16 p.m. Exquisite tetanus and opisthotonus in extension.

3.50 p.m. Tetanus and opisthotonus.

4.00 p.m. Tetanus and opisthotonus.

TABLE III
Tropaeolin 00

NUMBER OF EXPERIMENT	BANA SPECIES	WEIGHT	DOSE PER GRAM OF FROG	PLACE OF INJECTION	TIME FROM INJECTION TO OPERATION	OPERATION	TIME FROM OPERATION TO SYMPTOMS	PREMONITORY	SYMPTOMS	REMARKS
		grams	mg.				hrs. min.			
1	Pipiens	30	0.31	Abdominal sac	Previous operation	Anterior third cerebrum	35	Primary depression	Tetanus, legs in Z shaped position	Previously opera- ted day before
2	Pipiens	30	0.31	Abdominal sac	10	Anterior third cerebrum	26	None	Tetanus, legs in Z shaped position	Previously thred out
3	Pipiens	22	0.30	Abdominal sac	15	Anterior third cerebrum	10	Primary hyperexcit- ability	Tetanus opisthotonus	
4	Pipiens	22	0.30	Abdominal sac	Previous operation	Anterior third cerebrum	45	Extreme depression	Tetanus opisthotonus	
5	Pipiens	22	0.30	Abdominal sac	15	Anterior third cerebrum	1 40	Exceptionally	Convulsions	Very toxic
6	Pipiens	28	0.45	Abdominal sac	5	Anterior third cerebrum			Late tetanus	Probably due to incomplete ab- sorption
7	Pipiens	33	0.95	Abdominal sac		None	30	Slightly hyperexcita- ble	No tetanus	Death on follow- ing day
8	Pipiens	24	1.04	Abdominal sac	15	Anterior third cerebrum		Immediate depression	Very feeble convul- sions	Too large a dose
9	Pipiens	16	0.58	Abdominal sac	10	Anterior third cerebrum	10	Depressed	Very feeble convul- sions	Toxic
10	Pipiens	28	1.68	Abdominal sac	30	Anterior third cerebrum	15	Primary depression toxic	Tetanus	Toxic
11	Pipiens	18	1.62	Abdominal sac	18	Anterior third cerebrum	17	Depression for over 15 minutes	Then marked tetanus	
12	Pipiens	35	0.31	Dorsal sac	20	Anterior third cerebrum	50	Slight depression	Tetanus	
13	Pipiens	38	0.68	Dorsal sac	Previous operation	Anterior third cerebrum	8	Hyperexcitability	Tetanus	Operation 5 days before
14	Pipiens	12	0.65	Abdominal sac	18	Anterior third cerebrum	2		Convulsions	
15	Pipiens	17	0.64	Abdominal sac	Previous operation	Anterior third cerebrum	15	Hyperexcitability	Tetanus	
16	Pipiens	36	0.52	Dorsal sac	9	Anterior third cerebrum	7	Slight depression	Convulsions and te- tanus	
17	Clamata	23	0.40	Dorsal sac	12	None	2 10	Slightly toxic	No convulsions	Recovery
18	Clamata	23	0.40	Dorsal sac	12	Anterior third cerebrum	25	Slight depression	Convulsions	
19	Pipiens	16	0.19	Abdominal sac	10	Anterior third cerebrum			Convulsions	Legs in Z shaped position
20	Clamata	38	0.20	Dorsal sac	13	Anterior third cerebrum	45	Very slight depression	Convulsions and flexor tetanus	Small dose
21	Clamata	18	0.26	Dorsal sac	8	Anterior third cerebrum	7	Very slight depres- sion	Extensor tetanus	
22	Clamata	32	0.15	Dorsal sac	12	Anterior third cerebrum	5	Very slight depres- sion	Extensor tetanus	
23	Clamata	72	0.29	Dorsal sac	5	Anterior third cerebrum	33	Slight depression	Long continued te- tanic convulsions	
24	Clamata	45	0.20	Abdominal sac	7	Anterior third cerebrum	30		Extensor tetanus	
25	Clamata	28	0.28	Abdominal sac	Previous operation	Anterior third cerebrum	42	Exquisite	Extensor tetanus	

Control Experiment No. 25. An equivalent dose of the drug injected into an intact frog produces no convulsions.

The behavior of the dyes Basel I and Basel III is in every respect so similar to those already studied, that it is not necessary to give protocols of their actions here.

ANALYSIS AND DISCUSSION

Taking into consideration the above data together with those already published by Abel and Barbour, we thus find four compounds: acid-fuchsin, phenol-sulphone-phthalein, naphthol yellow, and tropaeolin 00—which all have a very similar pharmacological and physiological action on the frog. They all affect the nervous system, producing convulsions and tetanus, and they differ in their action from that of a large number of other convulsant drugs, in that this effect can be produced in a much shorter time after injection and by using much smaller doses if we divide the anterior third of the cerebrum from the rest of the brain. The effect is the same whether the drug be injected first and the cerebrum operated afterwards, or the brain be operated on first and the drug injected later.

By way of an explanation of the effect of the removal of the anterior third of the brain Barbour and Abel have expressed themselves as follows:¹²

The rapidity with which the convulsions follow upon the injury to the cerebral lobes under all circumstances make it appear permissible to assume that the convulsions are held in check in the intact acid-fuchsin frog as a result of inhibitory influences that proceed from the central lobe to subcortical centers. We need not necessarily conclude that the inhibitory influences are of a tonic character, or in other words, that such impulses continually pass from the cortex of the frog to lower centers, though we believe in the general statement that inhibition in the central nervous system is an integral part of the activity of its irritable constituents. We are quite content for the present to confine the occurrence of such inhibitory impulses to the case of frogs poisoned with acid fuchsin.

The passage just cited indicates the limitations that were made by Barbour and Abel in propounding their hypothesis.

¹² This Journal, vol. ii, no. 3, p. 197, 1910.

Joseph and Meltzer have raised a question of interest in this connection, and I here cite their words:¹³ "There are two conditions which are capable of greatly increasing the toxicity of acid-fuchsin for the frog, The removal of the anterior third of the brain (Barbour and Abel) and cardiectomy. . . . Is it possible to explain the convulsant action of both procedures by a single hypothesis?" Joseph and Meltzer were unable to offer an hypothesis which explained the convulsions of the two procedures. Professor Abel has shown (see p. 581) that the anomalous toxicity of acid-fuchsin after cardiectomy is best explained by the peculiar distribution of the drug and by the increased sensitiveness of the central nervous system in this case. Two frogs, one of which receives acid-fuchsin after a previous cardiectomy and the other of which receives acid-fuchsin after the previous removal of the anterior third of the brain, have two things in common, *convulsions and an equal content of acid-fuchsin in the brain and cord at the time of the convulsions*, when the minimum effective dose for each of these two procedures is used. Barbour and Abel stated that it required 0.35 mg. per gram of body weight to produce convulsions after partial ablation of the cerebral lobes. A smaller dose than this is however, effective, but the time that elapses before the convulsions appear, is greater than when the large dose is employed. I have found that the lower limit of the effective dose lies at or just above 0.2 mg. per gram of body weight and that this is the lowest dose of acid-fuchsin under which the spinal cord and brain will receive enough of the drug, with the cardiac mechanism intact, to give a definite pink color on the addition of hydrochloric acid. As was shown by Professor Abel this minimum quantity must always be present in the spinal cord and brain of the cardiectomized frogs also if convulsions are to be obtained.

While there is an agreement in respect to the *amount of acid-fuchsin present in the brain and spinal cord when convulsions are induced by the two procedures*, (cardiectomy and removal of the anterior third of the brain), there is no agreement in other respects.

Cardiectomy soon depresses the animal, and if the injection is made two hours after the cardiectomy no convulsions will be seen.

¹³ This Journal, vol. iii, no. 2, p. 202, 1911.

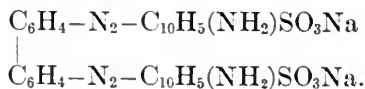
Removal of the anterior third of the brain, in itself, gives rise to no symptoms whatever. The frog thus operated upon behaved in every way like a normal frog. What is more, acid-fuchsin in doses of 0.35 mg. per gram of body weight,¹⁴ will still induce convulsions even when the anterior third of the brain has been removed a week prior to the injection of the drug.

Whether or not we attempt to give an explanation which assumes an inhibitory control of the lower centers of the cerebral lobes, such as was given in a guarded manner by Barbour and Abel, the fact remains that we are here dealing with a phenomenon which is seen only with a certain number of dye stuffs and not with other convulsants. For the present we are not able to show that ablation of the anterior third of the brain has a deleterious action on the oxygen intake of the spinal cord and brain, or in any other manner influences the metabolism of these organs, rendering them more sensitive (as does cardiectomy) to the action of acid-fuchsin. The phenomenon cannot be classed with the instances of anomalous toxicity following cardiectomy (Joseph and Meltzer) which have been discussed in detail by Professor Abel.

Another very interesting question arises as to whether the similarity of action of our four drugs is due to some chemical properties common to them as a whole. On this point we can only record the following observations:

In the first place all of our drugs are dyes soluble in water. In the second place, every one of the substances is taken up by the brain and cord, as could be demonstrated either by direct inspection or by means of chemical reagents.

Of those dyes which we tried and which were not taken up by the brain and spinal cord, none showed any convulsant action. Thus, for example, Congo-red,

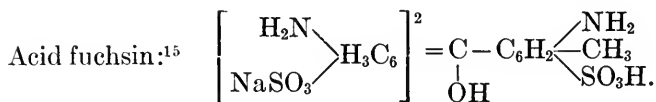
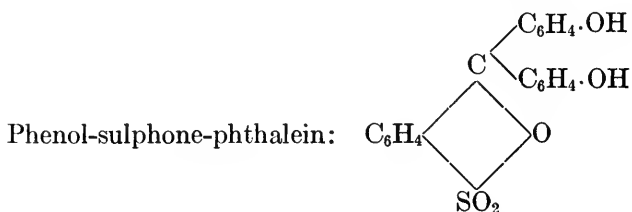


a dye freely soluble in water, can be injected into frogs in large quantities without any effect. It was found not to be absorbed

¹⁴ This Journal vol. ii, no. 3, p. 193, 1910.

by the nervous system. The same is true as far as our observations went of the dyes, methyl orange and scarlet red.

In the third place, on examining the chemical structure of our four bodies we note the following formulae:



It will be seen from the formulae that they are all sulphonated bodies. Furthermore we have noted that other dyes, which are not sulphonated will not produce convulsions even though they be taken up by the nervous system. Thus, for instance, methyl violet and carmine both are taken up by the nervous system, but do not produce convulsions. They are not sulphonated dyes, the formula of the first being $\text{C}_{19}\text{H}_{12} \cdot (\text{CH}_3)_5 \cdot \text{N}_3\text{HCl}$, that of the latter $\text{C}_{22}\text{H}_{22}\text{O}_{13}$. Methylene blue is a dye freely taken up by the nervous system, and containing sulphur, but the latter is combined in the molecule not in the form of a sulphone group SO_2 . It produces no convulsions whatever. Impressed by this interesting chemical relationship we looked for other sulphonated dyes, to see whether they too will give convulsions. Through the kindness of Dr. Evans of the Anatomical Laboratory of this University we obtained samples of the commercial dyes Basel I, Basel III, and Basel VIII. All of these were soluble in water. On injection into frogs, the dyes Basel I and Basel III, were found to behave exactly

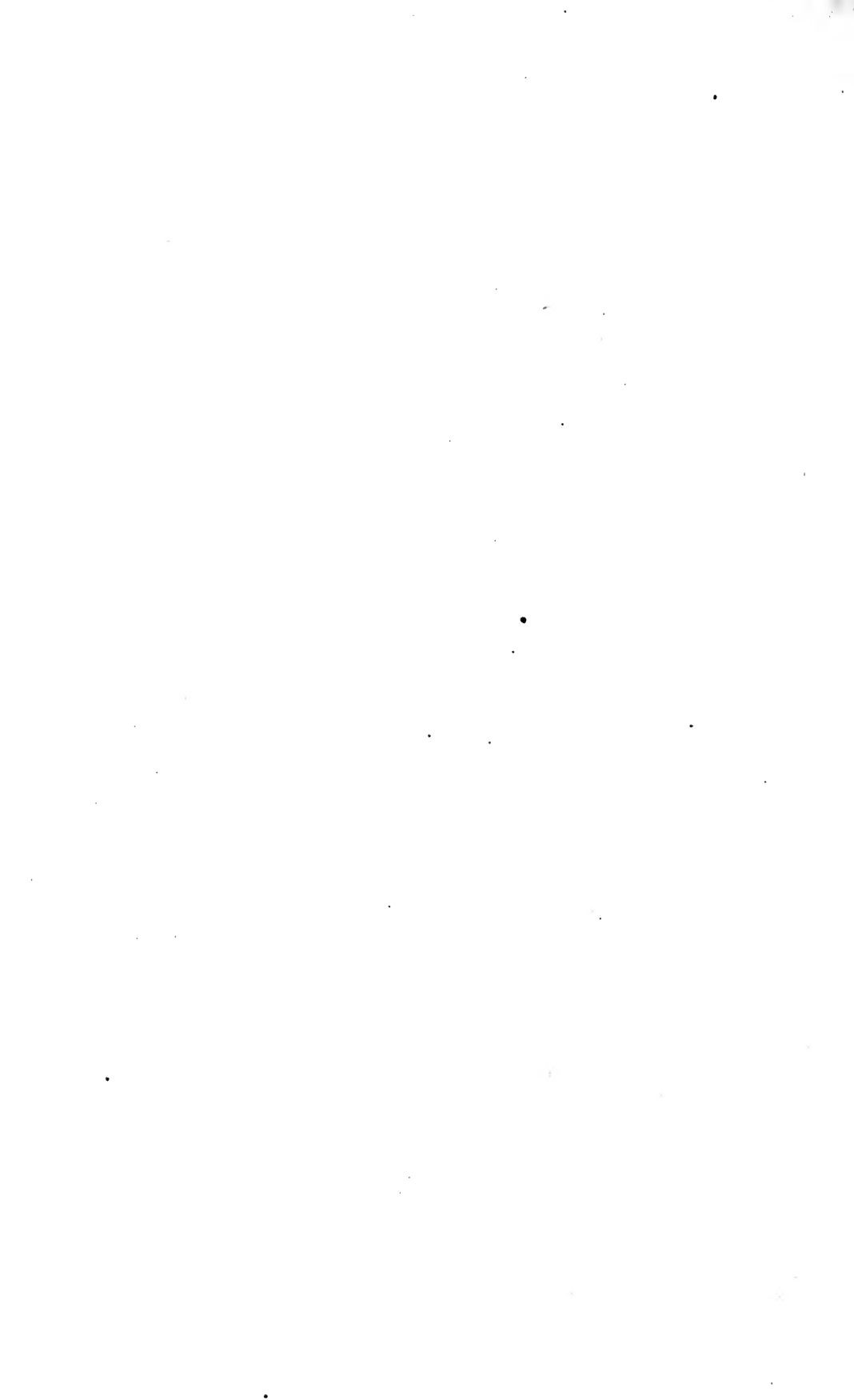
¹⁵ Leon Lefèvre, *Matières Colorantes*, vol. ii, p. 1064, 1896.

like tropaeolin 00 and the other convulsant dyes, that is, they produced convulsions and tetanus, and the same convulsions were produced much more quickly and by much smaller doses, in the decerebrate animals, the minimum dose of Basel I being only 0.25 mg. per gram of weight of frog, and that of Basel III being only 0.38 mg. per gram weight. Basel VIII did not produce any convulsions or tetanus either in intact, nor in decerebrate frogs. All three were taken up by the brain and spinal cord. On learning the constitution of these dyes later, it was found that Basel I and Basel III were both sulphonated bodies, whereas Basel VIII was not. It is very probable that a further search will reveal other sulphonated dyes with similar properties.

SUMMARY

Our observations lead us to the following conclusions:

1. The dyes sulphone-phenol-phthalein, naphthol yellow, tropaeolin 00, Basel I, and Basel III, behave like acid-fuchsin and when injected into frogs produce convulsions and tetanus.
2. The convulsions and tetanus can be produced much more quickly and by much smaller doses of these drugs, if the anterior third of the frog's cerebrum be cut off either before or after the injection.
3. In regard to their physical and chemical properties, these substances are all dyes, all soluble in water, are all taken up by the brain and spinal cord, and are all sulphonated bodies.



THE SEAT OF THE EMETIC ACTION OF APOMORPHINE

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The studies upon the pharmacology of the digitalis group which have been carried on in this laboratory¹ together with observations of the actions of these drugs on man² have long inclined us to the view that the vomiting seen in both their therapeutic and their pharmacologic applications is in all probability due to the direct action of these drugs upon the central vomiting mechanism rather than to a local irritant effect upon the stomach. In our efforts to establish the truth or the falsity of this conception it occurred to us that if we could show the parallelism between the phenomena and mode of action of a purely centrally acting emetic and the mode of action and phenomena of the digitalis bodies in the production of emesis we would have done much to confirm our beliefs.

Apomorphine is the only emetic in common use which is generally reputed to produce emesis by its direct central action alone, and it was chosen, therefore, as the drug with which to compare the digitalis bodies insofar as their emetic actions are concerned, but for reasons which will become apparent we undertook a preliminary research on apomorphine before proceeding with the comparison of the digitalis bodies, and we decided to present this pharmacologic study on apomorphine as a separate paper before publishing that on the seat of the digitalis emetic action.

¹ Hatcher and Bailey: Jour. Am. Med. Ass., 1910, lv, 1697.

² Bailey: Am. Jour. Med. Sc., 1911, cxlii, 183.

Before passing on, to the presentation of our own experiments on this problem in hand, it seems desirable to give a brief discussion of the various points brought forth in the literature and to present the existing arguments both for and against the view of the central action of apomorphine.

Following almost at once upon the discovery of the drug by Matthiesen and Wright in 1869, Gee³ remarked, “. . . it is clearly an emetic which does not act by causing direct gastric irritation (sub-inflammation), but which acts as blows upon the head, foul sights or smells, or mere imaginations act.” This is worthy of mention as an example of acute perception and close clinical observation rather than as a pharmacologic fact, which in reality it is.

The absence of irritant properties of apomorphine is against a local action of the drug on the stomach. Siebert⁴ gave daily emetic doses to dogs over periods of from four to six weeks and found on autopsy that there were no signs of any local irritation or inflammation and Reichert⁵ showed that solutions of the drug were not irritant to the conjunctiva or cornea when placed in the eyes of animals.

Siebert⁶ observed that larger doses were required to produce emesis when given by mouth than when given sub-cutaneously and thought this an argument in favor of central action. Reichert⁷ drew the same conclusions from his observations that the effect was produced regardless of the channel by which the drug was introduced into the body. A number of observers remarked that the action seemed to be more prompt after sub-cutaneous administration than after oral, and Reichert⁸ noted that large doses were not nearly so efficient in causing vomiting as were smaller ones and he expresses the opinion that from this, “It would seem as though in small doses the drug acts as a stimulant but in larger as a depressant to the vomiting centers.” Much

³ St. Barth. Hosp. Rep., Lond., 1869, v, 215.

⁴ Diss. Dorpat., 1871, also Arch. der Heilkunde, 1871, xii, 522.

⁵ Phil. Med. Times, 1879-80, ii, 254.

⁶ Loc. cit.

⁷ Loc. cit.

⁸ Loc. cit.

the same observations and conclusions were made by Harnack⁹ who remarked that while no vomiting followed very large doses, it was doubtful whether this was due to a paralysis of the center or not.

So unconvincing an argument as the close chemical similarity existing between apomorphine and its mother substance, morphine, has been offered as being in favor of its central action in the production of emesis. In this same category, though of much more weight, is the fact that all of the other direct actions of apomorphine excepting, of course, those resulting from purely local application, are exerted upon the several medullary centers or upon the higher cerebral areas.

Every one of the arguments thus far mentioned may be considered as lending some element of support to the view of central action but none can be regarded as conclusive. More direct evidence was sought along experimental lines and the rôle of the vagus was studied by many observers. Luettich¹⁰ observed that emesis in dogs did not occur when they were lying on their backs and it seems that Quehl who had found that section of the vagi¹¹ prevented emesis had restrained his animals in that position thus vitiating his result. Siebert¹² released his dogs after vagotomy and found that apomorphine did cause emesis. This occurrence of vomiting in dogs after vagotomy has been confirmed by many observers among whom may be mentioned Harnack¹³ Riegel¹⁴ David,¹⁵ and Choupe.¹⁶ To this evidence regarding the action of apomorphine after vagotomy may be added the findings of Openchowski¹⁷ that the vagi are the sole paths through which the purely peripheral reflex emetics, such as copper sulphate, act when introduced into the stomach.

⁹ Arch. f. exp. Path. u. Pharm., 1874, ii, 254.

¹⁰ Ueber den Mechanismus des Brechacts etc., Kiel, 1873; quoted by Harnack, loc cit.

¹¹ Quehl: Diss. Halle., quoted by Harnack.

¹² Loc. cit.

¹³ Loc. cit.

¹⁴ Quoted by Harnack.

¹⁵ Gaz. d. Paris, 1874, lii, 464.

¹⁶ Quoted by David.

¹⁷ Centbl. f. Phys., 1889-90, iii, 1.

More direct proof of the central action of apomorphine was sought by Reichert¹⁸ who ligated the thoracic aorta, thereby excluding the stomach from the circulation, and who then induced vomiting by giving the drug subcutaneously. He concluded from this experiment that, "The action of the drug could not be that of an effort at elimination causing stimulation of peripheral gastric nerves." Sollmann¹⁹ says, "After the blood vessels supplying the stomach have been ligatured, apomorphine does not produce vomiting when placed in this organ, but acts when injected into the general circulation (from which it cannot reach the stomach)." This latter argument is not conclusive evidence of central action for it fails to take into consideration the possibility of excretion and subsequent local action upon the intestines at some point below the stomach, and furthermore the wholly abnormal condition of the organ might be quite effective to prevent penetration of the drug sufficiently to stimulate the reflex mechanism even when it is put directly into the stomach. In addition we have found the traumatism caused by such ligatures and the abnormal state of the mucosa of the stomach frequently provocative of emesis without the aid of any drug.

Magendie²⁰ replaced the stomachs of dogs by pigs' bladders and was able to induce typical emesis thus excluding the possibility of any local reflex action from the stomach as the cause of the vomiting. This does not eliminate the possible reflex action arising from a portion of the intestine below the seat of the operation.

By transverse section of the spinal cord in the region of the first, to third cervical segments Gianuzzi²¹ was able to show that the vomiting center lay above this level. Starting with this information Thumas²² turned his attention to a direct location of the center. He cited the contention of Grimm²³ confirmed

¹⁸ Loc. cit.

¹⁹ Sollmann, Text-book of Pharmacology, 1906, 2d ed., p. 306.

²⁰ Quoted by Magnus, *Ergeb. d. Physiol.*, 1903, ii, 2, 645.

²¹ Quoted by Thumas: *vide infra*.

²² *Virch. Arch.*, 1891, cxxiii, 44.

²³ *Pflueger's Arch.*, 1871, iv, 205.

by Greve²⁴ who believed that the vomiting and respiratory centers were indential. Thumas agrees with Harnack that this contention is incorrect for there is no vomiting in deep narcosis although the respiratory center is then capable of stimulation by apomorphine. On this basis Thumas believed the vomiting center was an entity and probably lay in the medulla in the neighborhood of the respiratory center. He carried on a series of sections in the medulla to locate the center and found, by the exclusion of all other areas, that a longitudinal section in the mid-line of the medulla extending for 5 mm. through the calamus scriptorius, with a lateral extent of 1 mm. to either side of the middle line, completely prevented all vomiting movements but did not in any way affect respiration. Further, the direct application to this small area of amounts of apomorphine insufficient to cause any perceptible effect when applied elsewhere in the medulla, invariably caused emesis. He consequently believed that he had located the vomiting center and that it was either a twin center or a bilateral single center lying in the mid-line of the medulla, just beneath the surface, and in the region of the calamus scriptorius. Ospanchowki objects to the assumption that this is the site of the center and contends that Thumas's work merely shows that at this point are located the tracts which pass to and from the corpora quadrigemina, where Ospanchowski would have us believe the center is situated.

Having summarized the principal arguments advanced in support of the existence and location of the vomiting center a few points may be mentioned which seem to weigh against the belief in the central action of apomorphine. Schuetz²⁵ observed the development of strong reversed peristalsis in the excised stomachs of dogs under the influence of apomorphine and other emetics and believed that this local effect upon the movements of the organ had much to do with the induction of vomiting. Batelli²⁶ destroyed the validity of this view by showing that precisely the same phenomena could be seen in the excised stomach when

²⁴ Quoted by Thumas, loc. cit.

²⁵ Arch. f. exp. Path. u. Pharm., 1886, xxi, 341.

²⁶ Influence des Médicaments sur les Mouvements de l'Estomac, 1896, Geneva

no drug had been employed. Quehl's experiments with section of the vagi which we have cited above supported the idea of local action but, as we have seen, they have been refuted abundantly. Some have thought the action to be more prompt after introduction of the drug into the stomach than when otherwise administered. This observation is seen at once to be inaccurate if the drug be given intra-muscularly and by stomach.

Reichert²⁷ asserts that apomorphine can be found in the first vomitus even when given subcutaneously. This would seem to be strongly in favor of the possibility of a local action. But he does not so regard it, for he believes that another of his experiments in which he excluded the stomach from the circulation by ligation of the aorta, shows that such excretion is not necessary for the production of emesis by the drug. Further, Bongers²⁸ and Valenti²⁹ were unable to find any trace of the drug under these conditions.

In order to emphasize the strength of the general belief in the central action of apomorphine we will enumerate the several arguments and observations which form the foundation of the belief.

1. The chemical similarity to morphine.
2. The necessity for larger doses by mouth than when given otherwise.
3. The slower action when given by mouth than when otherwise given.
4. The lesser effects of large doses as compared with small.
5. The absence of all irritant properties when it is applied to mucous membranes or the cornea.
6. The fact that all of its other direct actions are upon the centers.
7. Its action when given by stomach after vagotomy.
8. Its action after ligation of the thoracic aorta which interferes with its excretion into the stomach.
9. Its action when minimal amounts are applied directly to Thumas's area in the medulla.

²⁷ Loc. cit.

²⁸ Arch. f. exp. Path. u. Pharm., 1894-95, xxxv, 415.

²⁹ Arch. di Farm. et Ther., 1901, ix, 233.

10. Absence of emesis after Thumas's section through this area.

For the sake of contrast we will likewise enumerate the points which have been raised against this view, all but one of which have been amply refuted.

1. The reversed peristalsis seen by Schuetz (completely refuted).

2. Absence of emesis after section of the vagi, observed by Quehl (completely refuted).

3. The contention that the drug acts more rapidly when put into the stomach than when given otherwise (abundantly refuted).

4. Presence of the drug in the first vomitus after subcutaneous administration (practically refuted).

While, as we said in the beginning of this paper, we have no absolute proof of the central action of the drug, the evidence when taken together is well nigh overwhelming in support of the occurrence of such direct central action.

Before passing on to a presentation of our own results in the study of this question we would draw attention to the difficulty of a definite determination of the seat of action in the case of apomorphine. This difficulty has been encountered by all who have made serious efforts to solve the problem, and if our chief experiment seems to be unnecessarily complex we can only say that we were unable to attack the problem successfully in any other way than that which we have employed and which involves the complete removal of the entire gastro-intestinal tract as devised by one of us (C.E.).

EXPERIMENTAL

For purposes of comparison it was necessary to establish the effective emetic doses of apomorphine by the various modes of administration. For this purpose, as well as in the case of all of the subsequent experiments recorded in this paper, we used a single specimen of the drug. In all cases the dose is expressed in milligrammes of the drug per kilogram of body weight of the animal.

The protocol of a single experiment of each type will suffice as the results of each series will be given in tabular form for easier analysis.

Determination of Emetic Dose by Stomach

Protocol of experiment:—June 27, 1911. Coach ♀, weight 8.6 kilo.
12:02 p.m. Apomorphine 0.25 mgm. per kilo. by stomach tube.
1:00 p.m. No emesis has resulted. Experiment closed.

The dose in the first experiment having proved ineffective, it was gradually increased until one was found which in most cases would cause vomiting. A record was kept of the time of the occurrence of emesis after the administration of the drug, as well as of the number of times the vomiting was repeated after a single dose. The results of this series are given in table I.

All animals were watched for at least an hour after the drug was given. The result in experiment 7 can be explained only on the ground of very great susceptibility on the part of the animal for no other dog vomited with a dose of less than five times that used in this case.

Taking these results we find that all of the dogs which received 5.5 and 6.0 mg. per kilo vomited but of the nine which had 5.0 mg. per kilo only four vomited. We have, therefore eight out of thirteen vomiting with a dose of from 5.0 to 6.0 mg. per kilo. Excluding the one anomalous result, after the dose of 1.0 mg. per kilo the interval which elapsed between administration of the drug and vomiting varied from four to ten minutes, with an average of seven minutes. The average of the doses causing emesis is 5.7 mg. per kilo. The concentration of the solution within wide limits seems to have no influence on the size of the dose or the rapidity of action.

Determination of the Effective Dose by Subcutaneous Injection

The drug was dissolved in 0.85 per cent saline solution fresh each day for the experiments.

TABLE I
Apomorphine to Dogs by Stomach Tube

EXPERIMENT	MILLIGRAMS PER KILOGRAM	EMESIS	INTERVAL BEFORE EMESIS	TIMES REPEATED
			<i>Minutes</i>	
1	0.25	0		
2	0.5	0		
3	0.5	0		
4	0.75	0		
5	0.75	0		
6	1.0	0		
7	1.0	+	22	0
8	2.0	0		
9	2.0	0		
10	3.0	0		
11	4.0	0		
12	4.0	0		
13	5.0	0		
14	5.0	0		
15	5.0	0		
16	5.0	0		
17	5.0	0		
18	5.0	+	10	1
19	5.0	+	4	1
20	5.0	+	8	1
21	5.0	+	6	0
22	5.5	+	6	0
23	6.0	+	8	0
24	6.0	+	6	0
25	6.0	+	8	3

Protocol—June 27, 1911. Experiment 13, tan dog, ♀.

2:06 p.m. Subcutaneous injection 0.5 mg. per kilo of apomorphine.

2:10 p.m. Vomiting. Repeated several times at brief intervals.

The results of the series are given in table II.

Emesis was not produced by a dose of less than 0.1 mg. per kilo by sub-cutaneous injection. Of the four dogs receiving this dose only one vomited. Of the two which had 0.15 mg. per kilo one vomited and the other did not. All of those which received 0.2 mg. per kilo or more vomited one or more times. By sub-cutaneous injection the effective dose of our sample of apomorphine would seem to be about 0.2 mg. per kilo. The interval between the giving of the dose and the onset of emesis is quite

variable. Excluding experiment 14, for reasons to be given, the limits of time range from two and one-half to ten minutes with an average of six and one-half minutes for the ten experiments. Experiment 14 is excluded because of the fact that the animal had a small dose of the drug by vein some four hours earlier and was still slightly depressed. The experiment was not intended for this series. Experiments 16 and 17 are worthy of note for they disprove the contention of Luetlich (p. 529) that

TABLE II
Apomorphine to Dogs, Subcutaneously

EXPERIMENT	MILLIGRAMS PER KILOGRAM	EMESIS	INTERVAL BEFORE EMESIS	TIMES REPEATED
			<i>Minutes</i>	
1	0.05	0		
2	0.05	0		
3	0.1	+	10	1
4	0.1	0		
5	0.1	0		
6	0.1	0		
7	0.15	0		
8	0.15	+	5½	1
9	0.2	+	3½	1
10	0.2	+	9½	2
11	0.25	+	10	Several
12	0.25	+	10	Several
13	0.5	+	4	Several
14	0.5	+	20	3
15	2.0	+	2½	Many
16	2.0	+	6	Many
17	2.0	+	4	Many

emesis does not occur when dogs are lying on their backs. In both of these experiments the animals were firmly restrained on their backs on boards and yet they both vomited quite promptly. It is, nevertheless, quite true that emesis is much less likely to occur *Determiner* these circumstances and has completely failed in some experiments in which we have tried it. The point is of little or no bearing in the present series of observations and is stated as a fact apart from the main trend of the work.

Determination of the Effective Dose by Intramuscular Injection

This was undertaken in the same general way as were the two previous determinations. The solutions were made fresh on the day when used and 0.85 per cent salt solution was the solvent.

Protocol—July 6, 1911. Experiment 5, tan dog, ♂, weight 7.2 kilo. 11:03 a.m. Intramuscular injection of apomorphine, 0.075 mg. per kilo.

11:07½ a.m. Vomiting, not repeated.

Table III gives the results of this series.

TABLE III
Apomorphine to Dogs, Intramuscularly

EXPERIMENT	MILLIGRAMS PER KILOGRAM	EMESIS	INTERVAL BEFORE EMESIS	TIMES REPEATED
			<i>Minutes</i>	
1	0.05	0		
2	0.05	0		
3	0.05	+	7	0
4	0.05	+	4½	1
5	0.075	+	4½	0
6	0.075	+	3	0
7	0.075	+	4	0
8	0.1	+	4	0

The results of this series are far more nearly uniform, partly at least, because our data were becoming greater and we had to do less feeling about to find our way. An analysis of this table along the same lines as the analyses of the preceding tables shows the average effective intramuscular dose to lie between 0.05 and 0.075 mg. per kilo with a very slight range of variation. The time interval before this dose causes emesis is also more nearly uniform and has a maximum range of three minutes with the average of eight experiments at four and one-half minutes. There is special interest in experiment number 7 in that it was made with a non-sterile solution prepared six weeks previously and not protected from light. That it was still as active as a freshly made solution tends to refute the notion which prevails to the effect that the drug rapidly deteriorates in solution. This old

solution was very deep green and very cloudy on shaking, in fact opaque, but was still up to its original strength after standing six weeks at room temperature. This is the only instance in the series of experiments recorded in this paper in which an old solution was used. This was the same solution as was used in experiment five.

Determination of the Effective Dose by Intravenous Administration

The problem of fixing this dose was fraught with some difficulty for it appeared that the rate of administration, the concentration of the solution, and such factors were frequent obstacles to the success of our earlier experiments. But to follow the same plan of presentation that we have used in the previous series the protocol of one experiment will be given, and then the table of results followed by a discussion of facts elicited.

Protocol—June 28, 1911. Experiment 16, dog, fox, ♂, restrained on back, quick incision, cannula inserted into femoral vein.

9:48½ a.m. Start intravenous injection.

9:51 a.m. Salivation, respiratory stimulation, followed at once by emesis.

10:30 a.m. Emesis was not repeated. Experiment closed and animal released.

Apomorphine injected = 0.039 mg. per kilo.

By the term "semi-dorsal" in the table we wish to indicate that the forward portion of the animal's body lies on one side while, for convenience of the insertion of a cannula into the femoral vein, the posterior extremities are placed much as though the animal were wholly on his back. From the table it will be seen that this change in position of the animals increased the proportion of successful injections. The position is decidedly a more comfortable one for the animals and obviates the added factor of uncertainty of emesis in the dorsal position, of which we have previously made mention. Anaesthesia interferes with emesis, hence the vein was exposed and the cannula or needle was inserted through a small incision, made quickly with a sharp knife,

and in no instance was there any sign of pain observed, for the animal would not even flinch, while being petted during the rapid cut through the skin.

TABLE IV
Apomorphine to Dogs, Intravenously

EXPERIMENT	MILLIGRAMS PER KILOGRAM	EMESIS	TIMES REPEATED	SALIVATION ONLY	POSITION OF DOG
1	0.22	0	0		On back.
2	0.172	+	0		Semi-dorsal
3	0.15	0	0	+	Semi-dorsal
4	0.148	0	0		Semi-dorsal
5	0.1	0	0	+	On back
6	0.099	0	0	+	Semi-dorsal
7	0.065	+	0		Semi-dorsal
8	0.061	+	0		Semi-dorsal
9	0.06	+	0		On back
10	0.05	+	1		Semi-dorsal
11	0.05	0	0	+	Semi-dorsal
12	0.05	+	0		Semi-dorsal
13	0.05	+	0		On back
14	0.043	+	4		Semi-dorsal
15	0.041	+	0		Semi-dorsal
16	0.039	+	0		On back
17	0.038	+	0		Semi-dorsal
18	0.037	+	0		Semi-dorsal
19	0.036	+	0		Semi-dorsal
20	0.036	+	0		Semi-dorsal
21	0.031	+	0		Semi-dorsal
22	0.029	0	0	+	On back
*23	6.0	0	0		On back

*Such a large dose was injected in the effort to determine whether it was possible to cause emesis by intravenous administration of a very large dose injected slowly after a smaller one has failed. No emesis occurred as the table shows.

Experiments 10 and 11 are bracketted to call attention to the fact that in these two cases only was the dose given a previously determined one. In these two the required amount of the drug was injected into the vein by hypodermic syringe. In all of the others of the series the dose was calculated from the quantity of a solution of a known strength which was run into the vein slowly and continuously until the appearance of the

usual symptoms of nausea indicated that emesis would follow. The figures given under the caption "Milligrams per Kilogram" indicate the smallest amount requisite to cause emesis in each case in which it appeared. In the instances in which there was no emesis the quantity is that given before closing the experiment.

The very large dose given in experiment 2 to cause emesis may be accounted for on the basis of the animal's having had two previous injections, and, as a result, having become depressed, or, refractory, as we prefer to call it. We will speak of this more in detail subsequently. This experiment may then be excluded from our computation of average dose, for all the other doses are single first doses. The average dose, based upon the fourteen experiments in which vomiting occurred, is 0.0455 mg. per kilo of dog weight. If we include the high figure of experiment 2 we raise the average effective vein dose to 0.053 mg. per kilo. In practice in the laboratory we have come to speak of 0.05 mg. as being the kilogram vein dose for dogs, that is for this single specimen of the drug.

While the effective dose falls within certain limits, the element of concentration influences considerably the success of intravenous administration and the accuracy of the determination of the dose required to cause vomiting. If the solution be too concentrated a very slight error in checking the injection at the earliest sign of impending emesis will make a very large error in the dose. On the other hand, if the solution be too dilute the rate of the injection will not permit of a sufficiently rapid administration of the drug to cause emesis with any degree of certainty. Further, it would seem that in many instances when the effective dose has been rapidly passed by moderate excess of the drug there arises a state of resistance to subsequent doses. That is, if the dose be exceeded moderately without the occurrence of emesis subsequent doses will then fail to cause emesis unless an interval of some hours is allowed to elapse. This condition we have come to designate as a "refractory state." It is obvious that the introduction of this element will be more frequent the higher the concentration of the solution used. For practical purposes

we have found that the most satisfactory concentration for intravenous administration is such that 1 cc. of solution contains 0.05 mg. of apomorphine; at least this is true for our sample of the drug.

We have referred to the earliest sign of impending emesis and would state that our experience leads us to believe that the first definite appearance of salivation and licking of the chops is a fairly trustworthy and nearly constant sign of the approach of vomiting. So early is it that in a few isolated experiments in which we have stopped the injection the moment the sign began to appear we saw these phenomena only and by subsequent injection in the same animal were able to show that we had stopped just below the effective emetic dose. For instance, in experiment 2 in the table on page 544 the animal vomited from doses of 0.038 and 0.042 mg. per kilo while he showed salivation only when the doses were 0.037 and 0.032 mg. per kilo. This sign of salivation is not absolutely constant but is so nearly so that it forms our best guide in these experiments as to when to check the injection.

The caption "Interval before emesis" was purposely omitted from table IV on account of the fact that the injection was continued until salivation appeared and, as we have seen, this may be considered to be the premonitory sign of the vomiting act. Emesis follows within one-half, to one and one-half, minutes after the first sign of salivation.

Accepting the average effective vein dose as being 0.045 mg. per kilo, we find that the extreme limits of variation are rather wide in isolated instances. The highest effective initial dose in our series was 0.065 mg. or 44 per cent above the average, while the lowest initial dose was 0.031 mg. or 32 per cent below. It is obvious, however, that here, as in other biological methods which aim at any degree of precision, standardization, for example, reasonably accurate results can be had as the result of the average of a sufficiently large series of experiments. We have used these observations and those given under the consideration of the intramuscular mode of administration as a means of detecting the presence of small quantities of the drug in various fluids.

The details of our methods will be considered in another place in this paper.

Some interesting observations were made on the effects of repeated intravenous doses of apomorphine to the same animal. The dogs in this series were all restrained in the semi-dorsal position described, and the solution was injected from a burette into the femoral vein. A condensed protocol of such an experiment is as follows:

August 26, 1911. Experiment 17, tan dog ♂, semi-dorsal position, no anaesthetic. Cannula in femoral vein. Solution of apomorphine in N/1 saline 1.0 cc. = 0.05 mg. of the drug.

11:12 a.m. Start injection.

11:13 a.m. Salivation; stop injection. 0.036 mg. per kilo apomorphine injected.

11:13½ a.m. Emesis.

11:16 a.m. Start injection.

11:17 a.m. Salivation. Stop injection. 0.0208 mg. per kilo apomorphine injected.

11:17½ Emesis

11:20 a.m. Start injection.

11:21 a.m. Salivation. Stop injection. 0.024 mg. per kilo apomorphine injected.

11:21½ Emesis. Repeated at 11:22¾.

11:24 a.m. Start injection.

11:25 a.m. Salivation; stop injection. 0.028 mg. per kilogram apomorphine injected.

11:25½ a.m. Emesis.

11:27 a.m. Animal released.

11:28 a.m. Diarrhoeal movement.

Table V presents the results of this group of experiments.

In table five the figures in the fourth column marked with an asterisk indicate the time from the cessation of the preceding injection as in these instances there was no emesis from which to take the time. The dose marked X in the second column under Experiment 5 which was given to the animal without causing emesis, owing to its similarity to the next following dose which did cause emesis, needs explanation. The reason for the

absence of emesis from the first of these two doses is that the drug was injected very slowly at this point to enable us to watch more carefully, if possible, the symptoms which the animal presented during the injection. As it presented none but salivation we gained nothing except a further confirmation of our previous observation that too slow an injection was not followed by emesis.

There is a noticeable lack of fatigue of the center as a result of the repeated injections. Two animals of the series vomited five consecutive times, and two, four times. In some of the experiments the limit of the number of repetitions was arbitrary and there is little doubt that the animals could have been made to vomit again. There is some tendency toward an increase in the size of the effective dose on repetition and where this occurs it is probably due to the development of a more or less noticeable general depression. In this as in the previous series, we were struck by the fact that in the majority of instances the administration of a dose moderately in excess of the effective one failed to result in emesis. In many of these there was no apparent depression to account for this lack of effect and in addition it was observed that a further increase in the dose was likewise ineffective (see also p. 540). We have thought that this might be due to the development of a refractory state of the center. In support of this view we have twice seen such a condition overcome by a rapid injection of a considerably larger dose with resulting vomiting. On comparing the details of these two experiments we found the large effective dose to be a little less than three times the previously effective small one, while in these and other experiments the dose causing the refractory state lay nearer to the small effective dose. We have offered this hypothesis upon the assumption that apomorphine acts upon the center. While we have termed this a "refractory" state it is quite probable that it is a condition of slight depression of the vomiting center *only* without the association of any obvious depression of other functions, and that the larger dose is therefore required to cause a sufficient stimulation, in the presence of this depression, to cause vomiting. To do this, also, the dose

TABLE V

Apomorphine to Dogs, Repeated Doses, Intravenous Injection

EXPERIMENT	MILLIGRAMS PER KILOGRAM	EMESIS	INTERVAL BE- TWEEN INJE- CTION AND LAST EMESIS	SALIVATION ONLY	EMESIS TIMES REPEATED
			<i>Minutes</i>		
1	0.036	+			0
	0.0208	+	2½		0
	0.024	+	2½		1
	0.028	+	1¼		0
2	0.038	+			0
	0.032	0	9¼	+	0
	0.042	+	*4¾		1
	0.037	0	5	+	0
3	0.037	+			0
	0.05	0	11		0
	0.037	0	*25	+	0
	0.047	+	*9		1
	0.039	+	4		0
	0.035	0	4½	+	0
	0.047	+	*5½		0
	0.038	+	3		0
4	0.061	+			0
	0.087	+	15		0
	0.076	0	15	+	0
5	0.039	0			0
	0.031	+	*4½		0
	0.045	0	5		0
	0.033	+	*5		0
	x0.055	0	5	+	0
	0.055	+	*5		0
	0.099	0	5½	+	0
6	0.041	+			0
	0.059	+	14½		0
	0.095	+	17		0
	0.085	+	1¼		0
	0.0785	+	4		0
7	0.044	0		+	0
	0.0675	0	*9½	+	0
	0.172	+	22		0

TABLE V—CONTINUED

EXPERIMENT	MILLIGRAMS PER KILOGRAM	EMESIS	INTERVAL BE- TWEEN INJEC- TION AND LAST EMESIS	SALIVATION ONLY	EMESIS TIMES REPEATED
			<i>Minutes</i>		
8	0.065	+			0
	0.0675	+	5		0
	0.180	+	16		0
9	0.043	+			4
	0.056	+	15		4
	0.069	+	19		many
	0.0385	+	13		many
10	0.036	+			0
	0.0577	+	14		0
	0.0657	+	15		0
	0.100	0	15	+	0
	0.087	0	*9		0
	0.160	0	*22		0
11	0.0525	+			0
	0.0557	+	10		0
	0.115	0	8		0
	0.065	0	*6½		0

must enter the circulation rapidly and reach the center in high concentration.

If this explanation is correct, and we hold it as such for want of one which is more satisfactory, it certainly lends support to the belief in the central action of apomorphine, for it seems improbable that mucous membranes could exhibit such phenomena. A still further confirmation of this explanation, as against that of general depression, is the fact that an intramuscular injection of two or three times the average intramuscular dose will also induce emesis after the refractory state has developed.

Having now completed the record of the details of our preliminary work, we may here summarize our observations and see what bearing they have upon the problem of the seat of action of apomorphine.

If the drug caused vomiting by local action on the nerve endings in the mucosa of the gastro-intestinal tract it should require

a smaller dose when introduced directly into the stomach than when it reached this organ or the intestine from the circulation. It should similarly act more promptly under the former than under the latter conditions. That neither of these is the case is shown most strikingly by a comparison of our results. The average effective dose by vein is 0.045 mg. per kilo while that by stomach is 5.7 mg. per kilo or somewhat over 125 times as great. Absorption from muscular tissue is known to be very nearly as rapid, and the effects nearly equal to those seen after intravenous injection in the case of many drugs, and with apomorphine we have found the average effective intramuscular dose to be very close to the intravenous. The dose by stomach tube is 95 times as great as the intramuscular and in this mode of administration we can compare the rapidity of action after administration. Emesis follows the intramuscular injection in four and one-half minutes on the average whereas it appears only after a lapse of seven minutes following administration by stomach, or is one and one-half times as long in coming on. Further, as we mentioned on page 534 the concentration of the solution when given by stomach tube effects neither the size of the dose required nor the rapidity of action. If the action were a local one concentration should influence both of these factors, for if the drug acted as an irritant the more concentrated the solution the more rapid the action and the smaller the amount needed. On the other hand high dilution would so reduce the intensity of the irritation as to delay the action and necessitate a larger dose. Even if the drug acted specifically on the nerve endings without being a general irritant concentration would of necessity influence both dose and rapidity of action. For a highly concentrated solution would not be distributed so quickly or so thoroughly over the mucous surface as would a dilute and hence would not so readily reach the structures upon which it acted. The decrease in size of the dose from that by stomach tube through the sub-cutaneous and intramuscular, to the intravenous is then but an indication of the degree and rate of absorption from the several structures concerned.

The uniformity of dose by a given channel of administration

certainly suggests very strongly that the action is upon a portion of the central nervous system, for we know of no other structure which might be concerned in the production of vomiting which reacts in such a constant manner to a chemical influence. The development of the refractory state, as we have seen, points strongly to the occurrence of central action.

All of these observations tend to support strongly the view that apomorphine acts directly upon the center and still more strongly to antagonize any idea of local reflex action. But these arguments are inconclusive and support the belief in the central action almost exclusively through elimination of the probability of the local. In spite of the seeming certainty that so small an amount of the drug as 0.045 mg. per kilo when given by vein could not cause emesis through excretion into the gastro-intestinal tract and subsequent local action when it required 125 times this amount to produce the same result if put directly into the stomach, it has not yet been proved conclusively that this is not the case.

Having the data upon which to construct a method of quantitative estimation of apomorphine by biological means, which is ten to fifty times as delicate as that of Reichert we proceeded to employ such a method in testing the vomitus for apomorphine. A condensed protocol will suffice to show our method of procedure.

Protocol—(1) Dog, ♂, semi-dorsal position. Apomorphine by vein, Weight 11.25 kg.

10:00 a.m. 100 cubic centimeters. HCl, 0.2 per cent by stomach tube.

10:05 a.m. }
to } Injected apomorphine—0.036 mg. per kilogram = 0.405
10:08 a.m. } mg. total.

10:09 Emesis. Vomited 85cc. fluid which was collected.

10:18 100 cc. HCl solution.

10:23 a.m. }
to } Injected apomorphine—0.0577 mg. per kilogram = 0.649
10:27 a.m. } mg. total

10:28 Vomited 5cc. fluid, collected.

10:38 100 cc. HCl solution

10:43 a.m. } Injected apomorphine—0.0657 mg. per kilogram =
to } 0.739 mg. total.
10:46 }
10:48 Vomited 165 cc. collected.
Total amount of apomorphine given to animal = 1.793 mg.
Total amount of vomitus collected = 255 cc.

Of the mixed vomitus 225 cc. (88.2 per cent) were taken and slowly concentrated at a low temperature on a water bath in a current of air and just neutralized with sodium carbonate. Made up to 25 cc. with saline.

(1a) Dog, ♀, weight 3.8 kg. semi-dorsal position. Intravenous injection.

1:34 p.m. Start injection of known solution of apomorphine.
1:39 a.m. Emesis. Apomorphine injected = 0.052 mg. per kilogram.
1:44 p.m. Start concentrated vomitus from (1) (88.2 per cent of the whole vomitus)
1:49 p.m. All of the concentrated vomitus run in without any symptoms and known solution of apomorphine has been started (1 cc. contains 0.05 mg. apomorphine).
1:51½ p.m. Emesis. Apomorphine injected = 0.055 mg. per kilogram.

Had there been as little apomorphine in the total vomitus as 0.05 mg. it would have amounted to 0.013 mg. per kilo for dog (1a) and this would have reduced even the larger dose of 0.055 mg. per kilogram to 0.042 mg. per kilogram but even with the total vomitus the second dose was slightly higher than the first. This quantity, 0.05 mg., is only one thirty-fifth of the total amount given to dog. 1.

(2) Control: To 25 cc. of the first vomitus from dog (1) we added 5.0 mg. of apomorphine and concentrated it to 9.5 cc. under the same conditions as obtained with the bulk of the vomitus. By intramuscular injections into dogs this concentrated solution was found to have lost little or none of its activity.

Similar results were obtained in a second experiment of this kind.

A third experiment was carried out briefly as follows. To a

dog weighing 12.6 kg. we gave 2.0 mg. of apomorphine per kg. (25.2 mg. total) intramuscularly and collected the vomitus in two parts, one the first vomitus, the other all the rest. These were strained and concentrated at a low heat as in the preceding experiments.

One-half of the total of the first vomitus was then injected intramuscularly into a dog weighing 6.5 kg. without causing any symptoms whatever. The emetic dose for this animal by intramuscular injection should have been between 0.05 and 0.075 mg. per kilogram, or about 0.4 mg. total. Hence there was less than 0.8 mg. in the whole of the first vomitus, or less than one-thirtieth of the total injected into the first dog.

One-half of the remaining vomitus was then injected intramuscularly into a dog weighing 4.3 kg. without any resulting symptoms. The amount which should be effective for a dog of this weight is under 0.3 mg. total. All of the remaining vomitus from the first dog therefore contained less than 0.6 mg. total, or less than one-fortieth of the amount injected into the original animal.

From these three experiments it is obvious that apomorphine is not excreted into the stomach of the dog in any considerable amount. The results of the experiments permit of but one deduction, that is; that apomorphine is not excreted into the dog's stomach in appreciable quantities in an *unchanged* form.

We now come to our last group of experiments, in which we will show that apomorphine can produce emesis quite independently of the existence of the gastro-intestinal tract.

Since emesis means literally the expulsion of the contents of the stomach through the oesophagus, it is obvious that no true emesis can occur in the absence of the stomach, but since the phenomena observed under the conditions to be described differ in no other perceptible way than in that of the expulsion of stomach contents from the emesis seen in the perfectly normal animal, we feel that we are justified in speaking of it as emesis in this connection. The phenomena usually result in the expulsion of mucus from the mouth and oesophagus precisely as in normal vomiting and the preceding phenomena, the retching with the contractions of the abdominal muscles, proceed exactly as in the

normal animal, so that we can say unhesitatingly *that no one who might see a normal dog and an eviscerated one vomit could distinguish the normal from the eviscerated animal*, so far as the vomiting act is concerned.

As the preceding discussion shows there is no simple means of proving that apomorphine acts on the vomiting center. We were therefore compelled to resort to the procedure to be detailed presently. In view of the well known fact that it is extremely difficult to reduce dogs to a condition of profound shock by injury to the intestines, it seemed probable that if a dog would survive the complete removal of its gastro-intestinal tract, such an eviscerated animal might be used in the proof of the central action of apomorphine.

The operation is as follows. Under careful light chloroform anaesthesia, with the animal in the dorsal position on an ordinary board, an incision is made in the median line extending nearly the entire length of the abdomen. The rectum is cut as close to the anus as possible, between two ligatures. A small vessel passes to the lowest part of the large intestine and this must be cut between ligatures. The large intestine can then be raised and the non-vascular peritoneal fold which attaches it to the posterior abdominal wall can be cut, freeing both large and small intestine up as far as the mesenteric vessels at the level of the kidneys. These vessels can be tied off in the form of a pedicle and cut between two ligatures, either at this time or as the last step in the operation. The stomach is now drawn down and two ligatures are thrown about it, the upper as close to the cardia as is possible. Cut between these two ligatures and free the stomach and spleen, which come away from their attachments together, until a double ligature can be placed about the gastric and splenic branches of the coeliac axis and these cut through. Should there now be any remaining vessels which have either not been tied or which have been tied but not cut they should be severed and the entire gastro-intestinal tract removed in a mass. The operation is very easy and can be done in a very few minutes after a little skill is acquired through practice. Our first few operations caused more shock to the dogs than was

seen in later experiments. In fact, in the later experiments there was often no shock of any severity for several hours. Chloroform was used to avoid the production of an excess of mucus and the tendency toward emesis which follow ether. It also requires much less chloroform than ether and the recovery is far more prompt. It is important that the narcosis shall not be too deep when the abdomen is opened, as the animal tends to succumb in this event. In the majority of cases the animals recover very well from the operation and seem but slightly depressed for an hour or more but subsequently sink into a state of deepening depression. If not killed they die in a few hours in tetanic convulsions. We give the protocol of such an experiment.

Protocol—December 7, 1911. Dog, ♀, on back on board.

9:49 Start anesthesia, chloroform.

9:53 Begin operation.

10:04 Stop anesthesia.

10:05 End operation. Changed animal to position on side.

11:45 Animal has been nearly conscious and seemingly about normal for some time.

11:47 Intramuscular injection of 1.0 mg. per kg. of apomorphine.

11:51 Emesis. Repeated once.

12:00 Experiment closed, animal killed with chloroform.

In experiments 6 and 7 in table VI the oesophagus together with the pharynx was cocainized as well as possible with 1 per cent cocaine solution on cotton on a catheter after washing out the mucus through a cannula tied into the gastric end of the oesophagus. Experiments 9 and 10 were also done with cocainization of the washed oesophagus. We washed in these two cases with a solution of sodium bicarbonate to remove the mucus more thoroughly and used cocaine in $\frac{1}{2}$ per cent solution. That the cocaine was acting on the oesophagus was evident from the disappearance of swallowing movements after a few minutes.

In the series of ten completely eviscerated dogs all vomited under the influence of apomorphine except two. In these two the depression as a result of the operation in one and of cocaine in the other was too great to permit of vomiting. The employ-

ment of cocaine was designed to exclude the possibility of apomorphine acting upon the nerve endings in the oesophagus and pharynx though the probability of the excretion of the drug into these regions is very slight. This absence of excretion into the oesophagus is supported by the previous experiments in which we were unable to find any of the drug in the vomitus. But, as it is possible for excretion to occur by unusual channels when it is prevented from taking place by its customary paths, we made the effort just described to exclude the action of the drug should it be so excreted.

TABLE VI
Apomorphine to Eviscerated Dogs, Intramuscularly

EX- PERI- MENT	DURATION ANESTHESIA	DURATION OPERATION	INTERVAL AFTER OPERATION	DOSE MILLIGRAMS PER KILOGRAM	EMESIS	INTER- VAL	REMARKS
	<i>Minutes</i>	<i>Minutes</i>	<i>Minutes</i>			<i>Minutes</i>	
1	12	12	43	0.1	+	2	
2	17	17	129	0.1	0		Depressed
3	19	16	42	0.15	+	2	
4	10	20	26	0.13	+	2½	
			254	0.13	+	3½	Same dog!
5	15	12	102	0.1	+	4	
6	5	14	72	0.1	+	3½	Oesophagus cocaineized
7	5	13	57	0.1	+	3	Oesophagus cocaineized
8	10+	18	21	0.1	+	7	
9	*13	11	55	0.1	0		Depressed. Oesophagus cocaineized
10	†9	8	62	0.4	+	2½	Oesophagus cocaineized

* It was thought before the apomorphine was injected that this animal would not vomit on account of marked depression and almost total general anaesthesia as a result of cocaine poisoning. Though we used only a one half per cent solution of cocaine in this and the next experiment we used a much larger quantity of it in order to be more certain of effects.

† The large dose of apomorphine used in this experiment was given to overcome the beginning depression arising from the cocaine.

Further evidence that the drug does not act reflexly from the oesophagus was furnished by the following experiment.

December 16, 1911. 11:23 a.m. Dog, ♀, eviscerated.

Had sufficiently recovered to permit of emesis sometime prior to noon. A cannula had been tied into the gastric end of the oesophagus.

- 12:05 Apomorphine, 6.0 mg. per kilogram in 30.0 cc. fluid was introduced into the oesophagus through the cannula and allowed to remain.
- 1:05 No symptoms have been produced by the apomorphine and animal is still about normal.
- 1:11 Upper end of oesophagus tied and animal killed with chloroform. Oesophagus removed entire with contents. Fluid carefully collected and mucosa washed with normal salt solution. Total brought up to 60.0 cc. Strained to remove a small amount of mucus which was present (about 5.0 cc.). After straining the mucus was washed with alcohol and the total fluid made up with saline to 100.0 cc. If there had been no apomorphine absorbed 1.0 cc. of this would then contain 0.5 mg. of the drug.

This was then tested for its apomorphine content as follows:

(1) Dog, ♂, weight 4.6 kg.

1:49 p.m. Injected intramuscularly 0.15 cc. of recovered fluid per kg.

1:54 p.m. Emesis.

Fluid injected theoretically contained 0.075 mg. apomorphine per kilogram of dog weight.

(2) Dog, ♂, weight 6.4 kg.

2:04 p.m. Injected 0.125 cc. per kilogram of recovered fluid intramuscularly.

2:10 p.m. Emesis.

Theoretical content of fluid injected = 0.62 mg. apomorphine per kilogram of weight.

(3) Dog, ♂, weight 4.4 kg.

1:55 p.m. Injected 0.1 cc. per kg. of recovered fluid, intramuscularly.

1:57 p.m. Slight salivation.

No emesis developed.

Theoretical apomorphine content of fluid injected = 0.05 mg. per kilogram of weight.

It was obvious from the lack of vomiting as a result of the presence of the apomorphine in the oesophagus for one hour that it caused no reflex stimulation of the vomiting center. The

second part of the experiment shows the lack of absorption from the oesophagus after so long a time, for the fluid recovered remained undiminished in strength as shown by the tests on dogs in which vomiting was produced by an amount of the fluid containing theoretically the average intramuscular dose of apomorphine per kg. of dog weight.

After we had undertaken to determine the possible participation of the oesophagus in the emetic action of apomorphine our attention was directed to the paper by Valenti³⁰ in which he describes the effects of cocainization of the pharynx and oesophagus of dogs with a 6 per cent solution of cocaine. This procedure prevented the expulsion of the stomach contents although there, 'Was a forcible endeavor to vomit.' He has shown, then, that in the intact animal apomorphine is quite capable of inducing the movements typical of the vomiting act after reflexes from the oesophagus and pharynx have been excluded. This confirms the results which we obtained in those evisceration experiments in which cocaine was used. Valenti's own conclusions relate solely to the question of the reflex inhibition of the muscular tone of the cardiac end of the stomach.

As a result of the second portion of our experimental work we have shown that apomorphine is not excreted in any considerable amount into the stomach in a free and unchanged condition. That apomorphine is effective in the production of vomiting after complete removal of the entire gastro-intestinal tract from the cardiac end of the oesophagus to the anus, and hence can cause its effect by action elsewhere than upon a portion of this structure. That it does not act upon the oesophagus in eviscerated dogs for it is effective after cocainization of this region and the pharynx, and that it neither acts locally upon the oesophagus when brought directly in to contact with its mucosa nor is it absorbed through the oesophageal mucous membrane.

We may now present the evidence brought forth as the result of our work in the following

³⁰ Arch. f. exp. Path. u. Pharm., 1910, lxiii, 118.

SUMMARY

1. Apomorphine acts in the smallest dose by intravenous injection, next when given intramuscularly, then sub-cutaneously, and the largest dose is required by stomach: This is to be taken as an indication that the drug acts only after its entrance into the circulation.

2. Vomiting occurs much more rapidly after intramuscular injection than when the drug is introduced directly into the stomach which is not in accordance with local action, but which rather implies a slower rate of absorption from the stomach than from the muscles.

3. Dilution within reasonable limits does not influence the size of the effective dose by stomach as would probably be the case if the action were a local one.

4. The uniformity of the dose by a given channel of introduction suggests that the action is upon a highly specialized structure such as a portion of the central nervous system.

5. The development of a "refractory state" by a slight excess during intravenous administration is not easily explained except upon the basis of direct central action.

6. The absence of the drug in a free state and unchanged in the vomitus is against the occurrence of a reflex action after excretion.

7. The removal of the gastro-intestinal tract does not prevent the production of emesis by apomorphine, hence the drug must act elsewhere than upon this structure.

8. After the removal of the gastro-intestinal tract and cocaineization of the oesophagus and pharynx apomorphine still causes typical vomiting movements. This is strong evidence that the drug does not act after vicarious excretion into the oesophagus.

9. The absence of emesis and of absorption from the oesophagus of eviscerated animals when apomorphine is allowed to remain in this structure for an hour would seem to exclude the occurrence of local action.

10. Absence of any evidence in favor of local action.

Upon the evidence to be obtained from the literature and that which we have presented from our own experimental observations we would draw the following

CONCLUSIONS

1. That the evidence in favor of the existence of a central controlling mechanism for the act of vomiting is overwhelming.
2. That all of the evidence favors the view that apomorphine acts directly upon such central mechanism.
3. That there is no valid evidence in favor of the local reflex action of apomorphine.
4. That apomorphine acts solely by direct stimulation of the central vomiting mechanism in the dog and probably also in man.

ON THE ACTION OF DRUGS AND THE FUNCTION OF THE ANTERIOR LYMPH HEARTS IN CARDIECTOMIZED FROGS

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Further work on the convulsant action of acid fuchsin and other dyes has, by the natural extension of research, brought me into immediate contact with the interesting observations of S. J. Meltzer and his pupils in regard to the distribution and the anomalous toxicity for cardiectomized frogs of acid fuchsin, morphine and other drugs. In commenting on his work Meltzer¹ says:

My experiments demonstrate that in the absence of the central circulation substances may be distributed through the body by a mechanism which in some instances may act even more promptly than the cardiac mechanism. In contradistinction to the central apparatus we may designate the distributory agent in question as the peripheral mechanism. The path of distribution employed by this mechanism can be nothing else than the tissue spaces.

It is made perfectly clear by Meltzer that he does not call upon the lymph hearts, or any other mechanical factors to furnish the driving force for this distribution for he states explicitly that drugs may be "efficiently," "fairly rapidly" and "satisfactorily distributed" throughout the entire body of frogs² whose cardio-vascular apparatus including lymphatics and lymph hearts

¹ Proceedings Royal Society, B, lxxxiv, p. 100, 1911.

² "Strychnine and adrenalin when injected into cardiectomized frogs are efficiently distributed all over the animal body and when administered in sufficient quantities produce the usual reactions of these alkaloids." Cited from S. J. Meltzer: The distribution of solutions in cardiectomized frogs. Journ. of Exp. Med., xiii, p. 557, 1911.

has been completely eliminated.”³ It is stated that “gravity does not play an essential part in this distribution,” also that “neither ciliary or other cell activities have anything to do with the migration of the solutions, since the distribution is efficiently accomplished also in animals dead and refrigerated for forty-eight hours” and that “the forces which are concerned in the distribution are probably diffusion and osmosis and perhaps also imbibition, capillarity, surface tension or even chemotaxis.”⁴

Some rather novel and striking effects are attributed to drugs when administered to cardiectomized frogs. Thus, it was found that “*the effects of morphine are much greater and incomparably more rapid than when administered to normal frogs.*”⁵ So too it was thought by Joseph and Meltzer that acid fuchsin, which “is efficiently distributed through the peripheral *lymph space mechanism*,” more easily produces convulsions in cardiectomized than in intact frogs, in short “affects the central nervous system much more strongly than when it is brought there by the normal circulation.”⁶ To illustrate, these authors have found that while in normal frogs doses of acid fuchsin varying from 1 to 4 mg. per gram of body weight are required to produce convulsions, in cardiectomized frogs so small a quantity as 1/40 mg. per gram will produce the same effect with certainty; also, while in the case of intact frogs the large dose (1 to 4 mg. per gram) of acid fuchsin requires from one to twenty hours for the development of the convulsions, in the case of the cardiectomized frogs the minute dose of 1/40 mg. per gram of frog brings on convulsions after an average interval of sixteen minutes.

In explanation of the fact that morphine and acid fuchsin act more powerfully and more rapidly upon the central nervous system of cardiectomized than of normal frogs, the following statements are made: “In the case of morphine we may assume that the circulating blood contains a secretion from one organ or from

³ Loc. cit., p. 548.

⁴ Loc. cit., p. 552.

⁵ Loc. cit., p. 557. Italics as in the original.

⁶ Joseph and Meltzer: On the convulsant action of acid fuchsin upon frogs deprived of their cardiac circulation. This Journal. iii. p. 201 1911.

several organs which is capable of modifying its actions upon the central nervous system. In the absence of the circulation the organs in question stop their secretion and the morphine is permitted to display its specific action without interference."⁷ "The blood receives from various organs of the body a variety of secretions, some of which, we know, are capable of neutralizing various toxins and poisons. It is therefore possible that it is some such substance in the blood which keeps down the toxicity of acid fuchsin in the normal animal. After elimination of the circulation and with it the neutralizing activity of the hypothetical substance, the acid fuchsin gets a chance to develop its toxicity."⁸ This hypothesis is then tested by experiments which apparently fully substantiate it.

To sum up briefly, Meltzer reaches the following conclusions: (1) "Animals possess a mechanism which is capable of distributing soluble substances through the entire body in the complete absence of the circulation. (2) Some substances when distributed by this mechanism act even more rapidly and efficiently than when distributed by the cardio-vascular mechanism."⁹ (3) The blood receives from various organs antitoxic substances which interfere with or greatly modify the specific action of such drugs as morphine or acid fuchsin. In cardiectomized frogs these neutralizing or detoxifying substances fail to interact with or reach the injected substances which are therefore capable of acting "much more strongly" upon certain organs than when brought to them by the "normal circulation."

The statement of Meltzer and of Joseph and Meltzer that acid fuchsin, strychnine and morphine are highly toxic for cardiectomized frogs when their solutions are injected into a subcutaneous truncal lymph sac, is easily substantiated and has been found to be entirely correct. Experiments have, however, been made by me which show that it is possible to give a different explanation for this fact from that offered by Meltzer. My experiments also suggest a

⁷ S. J. Meltzer: *Journ. Exp. Med.*, xiii, p. 552, 1911.

⁸ Joseph and Meltzer: *This Journal*, iii, p. 203, 1911.

⁹ *Journ. Exp. Med.*, xiii, p. 552, 1911.

restatement of the facts in regard to the distribution of drugs in the body of the cardiectomized frog and in regard to the agencies which effect this distribution.

DIFFERENCES IN DISTRIBUTION OF DRUGS IN CARDIECTOMIZED
AND IN NORMAL FROGS

Statement. When a solution of a drug is injected into one of the subcutaneous truncal lymph sacs of a cardiectomized frog, the solution, or its solute, reaches the subdural lymph space with relatively little loss to other parts of the body, and hence the drug is offered to the brain and spinal cord in relatively large amount, and these organs are therefore in a position to take up the drug freely by imbibition into their tissue spaces. On account of the elimination of the cardiac circulation, the drug is retained as it can not be carried away to other parts of the body that have a greater avidity for it. In contradistinction to this, a solution of the drug of equal or even of much greater strength which is injected into a frog with intact cardiac circulation, is so thoroughly distributed throughout the entire body of the animal and so largely bound to other tissues which have a greater avidity for it, that not enough is left at the disposal of the brain and cord to affect these organs physiologically. With drugs such as morphine and acid fuchsin the increased susceptibility of the central nervous system to their action which results from the absence of the circulation is also a factor (though one which has no relation to the peculiarities of distribution after cardiectomy) in lowering the dose required to produce convulsions.

By way of illustration we may take a concrete case in which acid fuchsin was used. Two frogs of approximately the same weight are selected (see protocols in the following section), the one (A) is cardiectomized, the other (B) is left intact. Frog A receives 1 or 2 mgs. of acid fuchsin (1 per cent solution in 0.65 per cent NaCl solution as used by Meltzer and Joseph) injected into the dorsal lymph sac. *In twenty minutes or less extensor convulsions have developed.* Frog B receives ten times the above quantity, that is 5 or 10 mgs. of the drug either in the form of a 1 per

cent solution as before, or in the form of a more concentrated solution in order that too large a bulk of fluid may not be injected. *No symptoms of any kind appear in this frog.*

The reason for the high toxicity of the drug in the case of frog A becomes evident at once when the brain and cord of both frogs are submitted to a simple chemical analysis. These organs are removed at a given time, say as soon as convulsions have made their appearance in frog A. At this time, which in the case of cardiectomized frogs is usually less than half an hour after the injections have been made, the loss of acid fuchsin from the normal frog B by way of the kidneys is not yet of material importance. When a *small quantity* of hydrochloric acid (10 per cent HCl in 40 per cent alcohol) is applied to the *crushed brain and spinal cord of the cardiectomized frog it will be seen that these tissues soon turn pink or even deep red*, while the brain and cord of frog B when similarly treated *show no trace of color*. The central nervous system of this frog has not absorbed enough of the acid fuchsin to be detected on the addition of acid, a very delicate test, and it is therefore evident why this animal does not have convulsions, even though it received five times as much of the dye as was given to the cardiectomized frog.

Further examination of the various tissues of both frogs by means of hydrochloric acid brings out the following facts. In the cardiectomized frog the acid fuchsin is found, aside from the brain and spinal cord, in the fasciae and muscles of the back, in the intervertebral cartilages, in the mucous membranes of the mouth, in the structures of the head and in the skin of the back. The tissues at the point of injection stain most deeply. Only a minute amount of the dye stuff is carried down into the lymph sacs of the legs and this is absorbed by the inner surface of the skin and the fasciae that cover the joints and muscles, even as far down as the toes (see fig. 1). The *interior* of the muscles of the four limbs is entirely devoid of the fuchsin. The violent extensor convulsions appear to play a part in driving the fuchsin solution down as far as the toes, for it was found in control experiments with frogs cardiectomized the day before and kept on ice wrapped in moist paper, that in this case the drug was carried down as far as the

knees only in the same length of time. The connective tissue of the fore limbs usually contains none of the dye, the stomach, intestines and other internal organs are likewise entirely devoid of it. It is therefore evident that the *limited distribution* of the acid fuchsin in the body of the cardiectomized frog makes it possible for the brain and spinal cord to receive enough of the dye stuff to bring on convulsions even when so small a dose as 1/40 mg. per gram of body weight is injected.

When the cardiac circulation is intact it requires a dose of acid fuchsin which is from ten to fourteen times larger ($\frac{1}{4}$ to 7/20 mg. per gram of body weight) than this minimal dose of 1/40 mg. before the brain and cord will absorb enough of the drug to show a pale pink color on the addition of hydrochloric acid.

An examination of the tissues of frog B, the frog with intact cardiac circulation, made with the help of hydrochloric acid as before, shows that here the acid fuchsin is distributed, though not uniformly,¹⁰ throughout the entire body of the animal, with the exception of the brain and cord as already stated, these organs not containing enough to be detected by the method employed. The fasciae, cartilages, muscles, internal organs as the stomach and intestines, the skin and other tissues that have a marked affinity for the dye, all stain a deep red when moistened with hydrochloric acid. It is apparent that when a given dose of the drug, say 5 to 10 mgs., is injected into a normal frog of average weight, say 40 grams, the tissues that have a greater binding power for the dye will receive the greater quantity and *the brain and cord under these conditions will not receive enough to be detected by the usual reagents*. A most striking instance of difference in binding power of two tissues is seen when the intervertebral cartilages and the connective tissue immediately surrounding the points of exit of spinal nerves are compared with the brain and spinal cord in

¹⁰ We have here another example of the lack of uniformity in the distribution of soluble substances in the tissues of normal animals, a phenomenon which is observed after intravenous injections, as after other methods of administration. I need only cite such well known instances of unequal distribution as are seen when fluorescein (Ehrlich, Nicati, Hamburger and others), salicylic acid (Jacoby and Bondi) or chloroform (Schmiedeberg) are administered.

586A

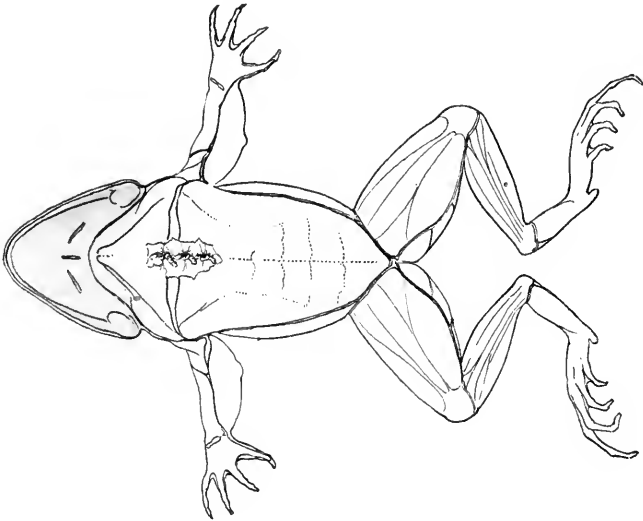


FIG. 3

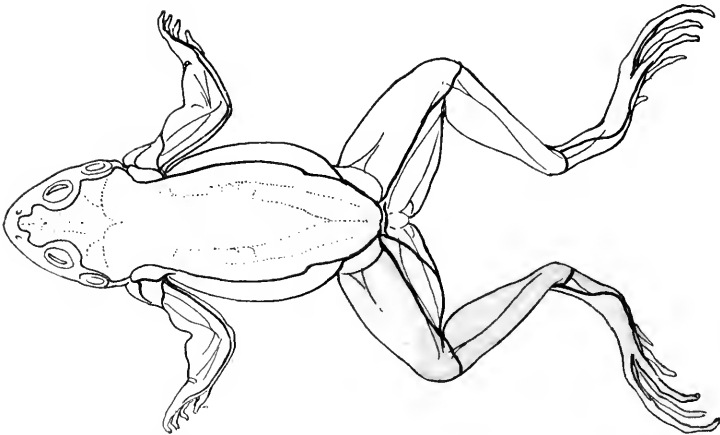


FIG. 2

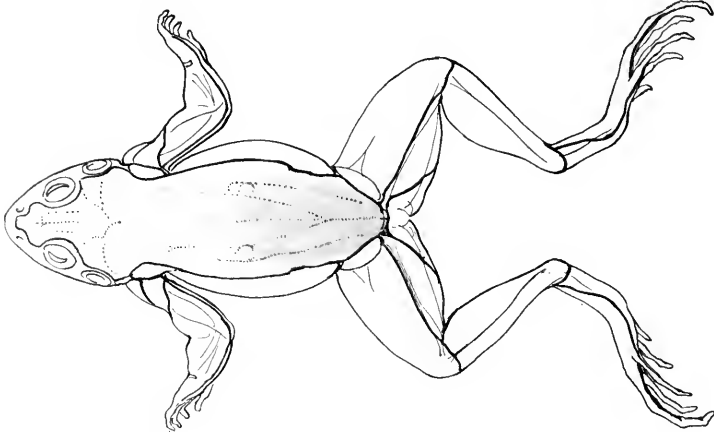


FIG. 1

respect to their content of acid fuchsin, as in the experiment just cited. The intervertebral cartilages and the tissues surrounding the points of exit of the spinal nerves take on a deep fuchsin red color on the addition of hydrochloric acid while the crushed central nervous tissue shows no trace of color.

It will be seen from the above and also from the protocols that follow that the distribution of a drug in cardiectomized frogs is limited to certain structures and regions of the body, being determined by the location and connections of the lymph sac into which the solution is injected, by the state of the lymph hearts, by the tension of the lymph sacs and by other factors such as the relative avidity of various tissues for the drug.

It is instructive to compare the tissues of several cardiectomized frogs that have each received the same quantity of acid fuchsin (1/20 mg. to 1/15 mg. per gram of body weight) the first frog (fig. 1) having received the solution into the lower part of the cranio-dorsal lymph sac, the second (fig. 2) into the middle of the left crural sac, and the third (fig. 3) into the upper part of the subvertebral sinus. In the accompanying figures it was attempted to depict the distribution of the dye stuff in the subcutaneous lymph sacs only. One hour after the injection the entire skin was stripped off and the body of each frog was immersed in a 2½ per cent solution of hydrochloric acid, immediately withdrawn, placed on filter paper and sketched.

Frogs 1 and 3 had convulsions very soon after the injection and in both animals the brain and spinal cord had taken up enough of the fuchsin to give a pink color on the addition of hydrochloric acid. The brain and spinal cord of frog 2 gave no indication of the presence of the drug and no symptom of any kind were noted in this case.

It will be seen that the most deeply colored tissues in figs. 1 and 2 are found at or near the site of the injection. The interior of the gastrocnemius and of the other large muscles of the left leg of frog 2 is entirely devoid of pigment; in the course of an hour the fuchsin is able to diffuse a short distance only.

In fig. 3 the area of most intense color is the floor of the submaxillary lymph sac, but it must be noted that the solution

was not injected at this point but into the deeply situated sub-vertebral sinus. The solution reaches the surface in the sub-maxillary lymph sac and in the anterior part of the cranio-dorsal sac, a fact which could not be shown in the drawing.

ACID FUCHSIN ONLY APPARENTLY MORE TOXIC FOR
CARDIECTOMIZED FROGS

From what has been said above it must be evident that acid fuchsin is only apparently more toxic for cardiectomized than for normal frogs.¹¹ In the experiments of Joseph and Meltzer the brain and spinal cord of cardiectomized frogs receive a large enough fraction of the small dose which is injected to impart a pink color to the brain and cord when these organs are treated with hydrochloric acid, while a large part of the tissues and muscles contain practically none of the dye. The "peripheral mechanism" is not capable, therefore, "of distributing soluble substances through the entire body in the absence of the circulation" as was supposed to be the case by Meltzer. In the intact frog, on the other hand, the dye stuff is distributed throughout the entire body of the animal and various structures such as tendons, fasciae, muscle tissue, cartilage, etc., which have a greater affinity for the drug than the central nervous system are enabled to compete with the latter, and when the quantity of the dye is insufficient to saturate all of these structures the brain and cord will not be able to secure the quantity that is needed to produce convulsions.

It must be emphasized in this connection that convulsions never occur after acid fuchsin unless the spinal cord (and brain) has absorbed enough of this substance to give a definite and unmistakable pink color on the addition of an acid to the crushed tissue. The

¹¹ This sentence may require a slight modification when the reasons for the long latent period that precedes the convulsions when acid fuchsin in doses of 1 mg. and over per gram of body weight is administered to normal frogs, are better understood. But it remains to be seen whether in this case the spinal cord and brain actually contain more of the dye at the time of the appearance of the convulsions, than is contained in these organs in the case of the cardiectomized or decerebrate frogs (Barbour and Abel).

tissues of the central nervous system have the same power as the muscles to neutralize acid fuchsin, that is, to turn it into a colorless salt and when only small or moderate quantities of this dye are used as in the experiments cited in this paper, the crushed brain and cord will give no evidence of its presence until an acid is applied.

In view of the fact that the brain and spinal cord of the cardiectomized frogs of Joseph and Meltzer which received from 1/20 to 1/40 mg. of acid fuchsin per gram of body weight were stained pink on the addition of acid, it will be more in accord with the principles of pharmacology to conclude that these frogs had convulsions because their central nervous system had absorbed a sufficient dose of the drug, than to invoke a special "neutralizing" substance¹² which ordinarily circulates in the blood and whose *absence* in the cardiectomized frog enables the acid fuchsin to set up convulsions. We are dealing here with a substance which has the properties of an acid and for its neutralization we need to invoke only the well known power of animal tissues to furnish hydroxyl ions. When the circulation is intact, the law of mass action and the relative affinities of the various tissues for the dye will determine how much each organ will finally obtain. With this goes hand in hand a constant though slow loss of the substance by excretion from the kidneys. It appears to me therefore that the results of Joseph and Meltzer are best explained on the basis of the color tests which I have made, all of which show that the *spinal cord and brain both of the cardiectomized and of the decerebrate frog must always contain a certain minimum amount of acid fuchsin before convulsions appear*. The cardiectomy may precede or succeed the administration of the fuchsin, just as the removal of the anterior third of the brain may precede or succeed the administration, but in neither case will convulsions occur unless the

¹² It has just been pointed out that acid fuchsin, in doses that suffice to cause convulsions, is in fact present in the brain and spinal cord *in the neutralized state* (neutral colorless salt) and only when a very much greater excess of the drug than is needed to produce convulsions is administered, do we find a part of it present in the unneutralized form. We see then that acid fuchsin is present in the central nervous system of the cardiectomized frog in the very form which according to Joseph and Meltzer should be relatively non-toxic.

requisite amount of the dye stuff—that amount which gives a pink color to the tissue—is present in the spinal cord. That so small a dose of acid fuchsin as $1/40$ mg. per gram of body weight suffices to furnish the brain and cord of the cardiectomized frog with this amount is due entirely, as has been shown by my experiments, to the peculiarities of distribution in this case.

CARDIECTOMY RENDERS THE CENTRAL NERVOUS SYSTEM MORE SUSCEPTIBLE TO THE CONVULSANT ACTION OF ACID FUCHSIN

While emphasis has been laid upon the fact that the central nervous system of cardiectomized frogs receives so large a fraction of the injected acid fuchsin in consequence of the peculiarities of distribution that inevitably follow cardiectomy, and while this has been described as the main reason for the apparently high toxicity of the drug in these animals, a second factor, already referred to, must not be lost sight of. This factor may be stated in the following words. The asphyxial condition of the central nervous system, or some alteration of state which is a direct consequence of the cessation of the circulation, renders this system more susceptible to the action of acid fuchsin.

The following experiment illustrates the point. Two frogs of about equal weight receive each $\frac{1}{2}$ to $\frac{1}{4}$ mg. of acid fuchsin per gram of body weight, by injection into the cranio-dorsal lymph sac. Fifteen minutes later one of the frogs is cardiectomized, the other being left untouched. In a short time the cardiectomized frog falls into convulsions while the intact frog gives no evidence of having received a drug of any kind.

It might be supposed that more of the fuchsin finds its way, in some manner, into the central nervous system of the cardiectomized frog. To test this hypothesis pairs of frogs of equal or nearly equal weight were injected with equal quantities of the drug, $\frac{1}{4}$ to $\frac{1}{2}$ mg. per gram of body weight. Half an hour or so later the lymph sac of each frog into which the injection was made was opened and well irrigated with 0.65 per cent solution of sodium chloride in order to preclude the possibility of transportation of acid fuchsin to the central nervous system *after* cardiectomy.

One of the frogs was now "cardiectomized" by the method of ligating, the other remaining untouched. In fifteen or twenty minutes convulsions occurred in the cardiectomized animal and now the spinal cord and brain of each frog were removed and tested with hydrochloric acid, care being taken that the same length of time elapsed in each case between the injection of the drug and the removal of these organs. After numerous experiments of this kind, it was not possible to conclude that the convulsions were due to a larger amount of acid fuchsin in the central nervous system of the cardiectomized frog. Sometimes the brain and cord of the cardiectomized frog were a shade more red than those of the control frog, but just as often the reverse was true. It seems rational, therefore, to conclude that cardiectomy brings on convulsions in frogs in whose bodies a certain dose of acid fuchsin has previously been distributed and whose central nervous system therefore contains a certain quantity of the dye, because the cellular elements involved have become more sensitive to the dye stuff. It is certain that of two frogs whose brain and spinal cord each stain to the same tint after the administration of acid fuchsin, the one that is cardiectomized will have convulsions, the other not. The increased sensitiveness of the central nervous system of the cardiectomized frog is the result no doubt of the lack of oxygen,¹³ coupled perhaps with the increase in carbon dioxide¹⁴ and other waste products. We can not very well assume that the increased sensitiveness is due to the lack of Meltzer's hypothetical antitoxic substance, since this has already had full opportunity to do its work, the cardiectomy having been performed subsequently to the distribution and neutralization of the fuchsin throughout the entire body of the frog.

Evidently the cessation of renal activity which follows the cardiectomy can not be made responsible for the convulsions, as in this event the brain and cord of the cardiectomized frog should

¹³ See A. Bethe: Vergleichende Untersuchungen über den Einfluss des Sauerstoffs auf die Reflexerregbarkeit. Festschr. f. J. Rosenthal, S. 245, Leipzig, 1906.

¹⁴ H. Fühner: Einige Beobachtungen an Erstickungsfröschen. Arch. f. d. ges. Physiol., Bd. 129, S. 255, 1909.

always contain more of the acid fuchsin than the corresponding structures of the intact animal. The proof, however, that loss of kidney function is not responsible for the convulsions is seen in the fact that nephrectomy does not produce them in the otherwise intact animal, at least not with doses approximating those above employed.

There can be little doubt that the facts here brought to light concerning the distribution of acid fuchsin in the body of the cardiectomized frog hold also, though with slight differences perhaps, for the distribution of strychnine, morphine and other drugs. The colloidal nature of a solution may be a matter of considerable importance in regard to its final distribution. Acid fuchsin, for example, is in the colloidal state in both acid and alkaline solution,¹⁵ while sulphate of strychnine and sulphate of morphine form non-colloidal aqueous solutions, as is well known. These alkaloids probably diffuse out of closed channels and lymph spaces with greater rapidity than does the colloidal acid fuchsin, and solutions of them which are equi-molecular with a solution of this dye stuff will likewise exert a lower osmotic pressure in consequence of their more ready passage through membranes. Differences in diffusibility are no doubt of importance in determining the final concentration of substances in a given tissue of cardiectomized frogs such as the spinal cord.

But the main factor in the rapid transportation of various drugs in cardiectomized frogs, from one lymph sac to others which communicate freely with it and from these again to others is the driving power of the lymph sacs, whether this be derived from the tension of their walls or from the surface energy of the injected solution or *from the pulsations of the surviving lymph hearts*. The molecular forces above referred to will, however, play the chief rôle when it comes to the penetration of an occasional membrane or to the final distribution in the tissue spaces of the skeletal muscles.

¹⁵ Wo. Ostwald: Kolloidchemie der Indikatoren. Zeitschr. f. Chem. u. Industrie der Kolloide, x, 95, 1912.

EXPLANATION SUGGESTED FOR THE GREATER TOXICITY OF
MORPHINE IN THE CARDIECTOMIZED FROG

Meltzer has shown that 6 or 8 mg. of sulphate of morphine suffice, when injected into the dorsal lymph sac of a cardiectomized frog, to develop a tetanus which comes on in forty or fifty minutes after the injection. In the intact frog it requires 10 mg. or more to produce tetanus, the so-called "late tetanus" which appears only after the lapse of several days. In explanation of the fact that the tetanus appears so quickly in the cardiectomized animal and after a smaller dose, Meltzer offers the theory here as with acid fuchsin, that the central circulation receives neutralizing substances from various organs and tissues which ordinarily modify the action of morphine. In cardiectomized frogs these neutralizing substances can not come into contact with the injected morphine which therefore now exerts its specific toxic action unhindered. Much is known in regard to the destruction of morphine in the tissues of warm-blooded animals at least, but for the explanation of Meltzer's observation no appeal need be made either to the well known slow oxidizing powers of the living tissue or to hypothetical antitoxic substances in the blood. Would it not be more in accordance with the facts to assume that here also the limited distribution of the morphine and its relatively high concentration in the brain and cord are the main reasons for its effectiveness in causing convulsions in small doses in the cardiectomized frog, though here also as in the case of acid fuchsin, we have probably to deal with an increased excitability of the central nervous system which is due primarily to absence of the circulation.

There are other points that have been emphasized by Joseph and Meltzer which are also explainable in the above manner. Thus it was found by these authors that so small a quantity of acid fuchsin as 1/125 mg. per gram of body weight, when it is injected into the aorta, suffices to cause convulsions in the cardiectomized frog, and this fact was put forward as furnishing striking evidence for the theory that "it is the blood of the cardiac circulation which reduces the toxicity of fuchsin."¹⁶

¹⁶ Joseph and Meltzer: *This Journal*, ii, 199, 1911.

On repeating this experiment I find that the small quantity of 1/125 mg. of acid fuchsin per gram of body weight just suffices to saturate the brain and spinal cord of the cardiectomized frog with the minimum convulsant dose of the drug. The crushed brain and cord in this case give, when treated with hydrochloric acid, the pink color¹⁷ which is always obtainable when cardiectomized or decerebrate frogs have convulsions after acid fuchsin. It seems logical, therefore, to bring this instance also into line with the other examples of anomalous toxicity discussed above. As in all the other instances of convulsions after acid fuchsin in cardiectomized frogs, so here also the spinal cord and brain receive a convulsant dose of the drug, a quantity which, in the absence of the distributing central circulation, is retained and necessarily exerts its pharmacological action.

PROTOCOLS OF EXPERIMENTS

Many experiments have been made to substantiate the statements made in the preceding pages, but I consider it unnecessary to give more than a few illustrative protocols, especially in view of the fact that further evidence for these statements will be found in the following paper.

Experiments which show that the central nervous system of the cardiectomized frog receives a much larger proportion of the injected acid fuchsin than does that of the intact frog

Experiment 1. *Rana clamata*, ♀; weight 50 grams. The spawn weighed 6.8 grams, giving 43.2 grams as the effective weight for comparison with male frogs since acid fuchsin is not taken up by the eggs. Heart ligated at 11.10 a.m. It may here be stated that in most of my experiments the action of the heart was eliminated by the method of ligature. A moistened glass rod is inserted into the mouth of the animal

¹⁷ One is obliged to wait a little longer for the development of the pink color in this experiment than in an experiment in which the fuchsin is distributed by the route of the lymph sacs. I conclude that the minimum effective quantity of dye stuff is more evenly distributed throughout the entire brain and cord, in the experiment under discussion, and only becomes visible as the acid penetrates the crushed tissue.

and pushed well down into the stomach. This raises the heart and it is then an easy matter to ligate this organ and the great vessels with curved needles of the proper size as Meltzer has pointed out. I always pass never less than three, and often four, ligatures around the heart starting with the first ligature at a point just below the clavicle and then working downward. This method is bloodless, much quicker than the method of excision and equally effective.

At 11.13 a.m. injected 0.2 cc. of a 1 per cent solution of acid fuchsin in 0.65 per cent NaCl solution (Joseph and Meltzer) into the middle portion of the cranio-dorsal lymph = a little less than $1/20$ mg. per gram of weight body.

In making the injections described in this and in the following paper I used a tuberculin syringe graduated to 0.02 cc. and armed with a very long needle. This makes it possible to go through the muscles of the thigh and then up to the middle of the dorsal lymph sac. When the injection is made in this way there is no leakage after withdrawal of the needle.

First convulsion at 11.54 a.m. The convulsions were allowed to continue until 11.58 a.m. when the entire bony canal containing the spinal cord and brain was quickly separated from the body. The brain and cord were then removed entire and placed on smooth filter paper, the vascular membrane surrounding the spinal cord, medulla and the optic lobes was removed and then the entire brain and cord were gently crushed so that a layer of nervous tissue about half a millimeter in thickness resulted. A quantity of a 10 per cent solution of hydrochloric acid in 40 per cent alcohol which just sufficed to moisten the crushed brain and cord was then applied. In a few moments the entire cerebro-spinal tissue took on a fine pink color. The intervertebral cartilages, the connective tissue at the points of exit of the spinal and cerebral nerves, the fasciae and the superficial and deep muscles of the back, the submucosa of the entire mouth, the tissues of the subscapular region and head, all showed the presence of the dye when tested in this way. It was not necessary to use acid to find the fuchsin in the fasciae and muscles lying immediately under the cranio-dorsal lymph sac. The deep red color of this entire region shows that more of the dye remains here than can be neutralized in the tissues. The internal organs, as the stomach and intestines, the anterior muscles of the abdominal walls, the muscles of the four extremities were entirely devoid of fuchsin. A little of the drug was, however, carried down into the lymph sacs of the legs and is taken up by the tendons and by the fasciae over the joints and

in part by those that cover the large muscles, as the lower half the gastrocnemius, for example, turned pink on the addition of acid. On cross section it was seen, however, that *none of the leg muscles contained any of the fuchsin in their interior*, the small quantity of the drug present being located entirely on the surface.

Experiment 2. *Rana clamata*, ♂; weight 45.7 grams. At 11.30 a.m. injected into the dorsal lymph sac 1 cc. of a 1 per cent solution of acid fuchsin in 0.65 per cent NaCl = nearly five times the quantity given to the cardiectomized frog in experiment 1. No symptoms of any kind. At 12.05 p.m. thirty-five minutes after the injection (this being the interval that elapsed in the case of the cardiectomized frog in experiment 1 the brain and cord were removed as in experiment 1 and tested with hydrochloric acid. Results negative. Everywhere else throughout the body the drug could be found. In this animal also, although the substance of the brain and spinal cord had taken up none of the dye, the intervertebral cartilages and the connective tissue in the neighborhood of the bony canal stained deeply on the addition of acid. The internal organs, as the kidneys, the stomach and intestines, the entire skin, all of the fasciae, tendons and aponeuroses of the body, the entire substance of all of the muscles of the body, the submucous tissues, all contained the dye, some tissues as fasciae and cartilages containing more than others and taking on an intense red color on the addition of the acid. In this case also, the region lying immediately under the dorsal lymph sac stained somewhat more deeply than other portions of the body, showing that even with the circulation intact an equal distribution is not attained in half an hour when a large quantity of the fuchsin is injected.

Experiment 3. *Rana pipiens*, ♂; weight 20.5 grams. Heart ligated at 11.41 a.m. Injected into dorsal lymph sac 0.15 cc. of the solution of acid fuchsin used in experiments 1 and 2 at 11.46 a.m. = about 1/15 mg. per gram of body weight. The frog was not examined until 11.59 a.m. when it was found stretched out in tetanus. Brain and cord removed nineteen minutes after injection, crushed and treated with hydrochloric acid, assumed a pink color. The rest of the body showed the partial distribution already noted under experiment 1. Here, however, the inner surface of the skin of the fore limbs and some of the tendons and fasciae of these limbs also assumed a light pink color on being tested with acid.

Experiment 4. *Rana pipiens*, ♀; weight 31.2 grams. Spawn weighs 6 grams giving 25.2 grams as the effective weight. Heart tied

off at 2.44 p.m. At 2.47 p.m. injected the minute dose of 0.1 cc. of 0.5 per cent solution of acid fuchsin in 0.65 per cent NaCl into the upper part of the prevertebral lymph sac, passing through the basilar lymph sac. Dose = $1/50$ mg. per gram of body weight. Convulsions began at 3.13 p.m. At 3.18 p. m. brain and cord removed, and legs cut off at thigh. Brain and cord showed the presence of the drug, turned pink on the addition of acid. Distribution in the body of this frog limited to the structures of the trunk. When double the above quantity is injected into the prevertebral lymph sinus of a cardiectomized frog, a small part of the drug may be carried by subcutaneous sacs as far down as the tips of the toes, and in this case the brain will be so quickly saturated with the drug that convulsions will appear in less than three minutes.

Experiment 5. *Rana pipiens*, ♀; weight, 39.8 grams. Spawn weighs 7.1 grams, so effective weight = 32.7 grams. Heart ligated at 4.14 p.m. At 4.17 p.m. injected 0.16 cc. of 0.5 per cent solution of acid fuchsin in 0.65 per cent NaCl into dorsal lymph sac = little less than $1/40$ mg. per gram of body weight. Convulsions appeared at 4.40 p.m. Brain and cord stained pink when treated with hydrochloric acid while rest of the body showed the partial distribution already described.

Experiment 6. *Rana pipiens*, ♂; weight 33.5 grams. At 3.19 p.m. injected into the dorsal lymph sac 0.5 cc. of a 1 per cent solution of acid fuchsin = 0.15 mg. per gram of body weight. No symptoms whatever. At 3.45 p.m. removed the brain and spinal cord and tested them with hydrochloric acid but without finding a trace of pink color. The intervertebral cartilages showed a pink color on the addition of the acid and the distribution throughout the rest of the body was as described in experiment 2. It will be seen that the intact frog in this experiment received six times as much of the dye per gram of body weight as the frog in experiment 5, and seven times as much as the frog in experiment 4, and yet no traces of it could be detected in the spinal cord and brain by the method here employed.

Experiments to show that cardiectomy increases the sensitiveness of the central nervous system toward acid fuchsin

Experiment 1. *Rana pipiens*, ♂; weight 24.5 grams. At 3.05 p.m. injected into the abdominal lymph sac, passing the needle from below through muscles of the thigh, 0.16 cc. of a 5 per cent solution of acid fuchsin = 0.33 mg. per gram of body weight. At 3.48 p.m. the abdominal lymph sac was opened and washed out with a 0.65 per cent solution

of NaCl, for the purpose of removing any unabsorbed fuchsin. The fuchsin had, however, been entirely absorbed from the lymph sac. At 3.50 p.m. ligature of heart. At 4.05 p.m. violent extensor tetanus. At 4.25 p.m. the frog still responds with tetanus when skin is irritated.

Experiment 2. *Rana pipiens*, ♂; weight 27 grams. At 3.07 p.m. injected 0.30 cc. of a 5 per cent solution of acid fuchsin into the abdominal lymph sac = 0.55 mg. per gram of body weight. At 3.53 p.m. heart was tied off. Convulsions began at 4.03 p.m. The frog remained responsive to stimulation 4.50 p.m.

Experiment 3. *Rana clamata*, ♂; weight 24.5 grams. At 11.35 a.m. injected into dorsal lymph sac 0.16 cc. of a 5 per cent solution of acid fuchsin = 0.33 mg. per gram of body weight. At 12.34 p.m. tied off heart, after first washing out the dorsal lymph sac with 0.65 NaCl solution. At 12.57 extensor tetanus. At 1.06 p.m. still falls into violent extensor convulsions when the table is jarred. Brain and cord removed at 1.10 p.m.; when tested with hydrochloric acid their crushed tissue assumes a light pink color.

Experiment 4. *Rana clamata*, ♂; weight, 25 grams. At 11.31 a.m. injected into dorsal lymph sac 0.15 cc. of a 5 per cent solution of acid fuchsin = 0.3 mg. per gram of body weight. At 12.38 p.m. the heart is tied off, after first washing out the dorsal lymph sac with 0.65 per cent NaCl solution. Extensor convulsions begin at 12.58 p.m. when table is jarred. At 1.12 p.m. brain and cord removed and tested with hydrochloric acid—both show a pink color.

More experiments of this kind were made with large as well as with small doses, and it was found that the lower limit of the convulsant dose of acid fuchsin for frogs of average weight cardiectomized *subsequently to the distribution of the acid fuchsin in the body* was 0.2 mg. per gram of body weight.¹⁸ Only when this quantity is injected into the intact frog does the brain and cord receive enough of the fuchsin to show a pale pink color on the addition of hydrochloric acid, and only then can convulsions be induced by unusual procedures such as cardiectomy or removal of the anterior third of the brain. Under normal conditions this minimum quantity in the brain and cord does not induce convulsions.

¹⁸ Macht has shown (see the preceding paper), that this is also the lower limit of the effective convulsant dose of acid fuchsin, for frogs the anterior third of whose cerebral lobes is removed subsequently to the distribution of the drug in the body.

ON THE PART PLAYED BY THE ANTERIOR LYMPH HEARTS IN THE DISTRIBUTION OF DRUGS IN CARDIECTOMIZED FROGS¹⁹

One of the first questions that naturally presents itself in taking up the study of the distribution of solutions in cardiectomized frogs pertains to the possible action of the lymph hearts in assisting in the distribution. Meltzer has come to the conclusion that "the lymph hearts can not assist in the distribution of solutions, except as aids to the cardiac circulation" and that "when the heart is ligated and cut out, the activity of the cardio-vascular apparatus, including lymphatics and lymph hearts is completely eliminated."²⁰

He also states that "the distribution is efficiently accomplished in animals dead and refrigerated for forty-eight hours." Inasmuch as the lymph hearts cease beating²¹ in about an hour after cardiectomy, it is apparent that whatever distribution is seen after this time is to be attributed solely to such agencies as capillarity, hydrostatic pressure (distension of lymph sacs, weight of the frog's body resting on the lymph sac) and diffusion.

While it is true that the distribution of solutions by non-vital agencies takes place both in dead frogs and in those that are cardiectomized just before the injection, yet the appearance of convulsions in Meltzer's experiments depends entirely on the integrity of the anterior lymph hearts. If these organs have ceased to pulsate, or if they have been excised or destroyed with the cautery following cardiectomy, convulsions will not be seen after the injection of acid fuchsin, strychnine or morphine.

The central nervous system does not retain its irritability very long if the operation of cardiectomy is promptly followed by destruction of the anterior lymph hearts. These organs carry on an independent circulation in the central nervous system for an hour or more after the blood circulation has been eliminated.

I shall not here discuss the anatomical relations of the communicating system of lymph sacs and sinuses and the anterior lymph

¹⁹ The following sections of this paper have been added since the foregoing part, which was written two months ago, was sent to press.

²⁰ Journ. of Exp. Medicine, vol. xiii, p. 548, 1911.

²¹ See Priestley, Journ. of Physiol., vol i p. 1, 1878-9.

hearts of the frog further than to remind the reader that these lymph hearts force their contents, each into its corresponding vertebral vein. The vertebral veins empty into the internal jugular veins which carry away the blood from the network of veins in the interior of the skull and spinal column. When the passage from the internal jugular vein to the innominate vein and thence to the *sinus venosus* is blocked by ligature of the heart, the pulsating lymph hearts force their contents backward into the venous capillaries of the highly vascular membranes of the central nervous system. Whether this is the only path by which a solution can reach the central nervous system from the truncal lymph sacs I am as yet unprepared to say, as there is still much to be learned concerning the relations of the lymph hearts, lymph sacs and lymph sinuses in the frog.

I assume for the present that the back flow from the actively pulsating anterior lymph hearts through the veins of the central nervous system and its membranes is the main channel by which drugs which are injected into a subcutaneous or deeply situated truncal lymph sac reach the spinal cord and brain of the cardiectomized frog. It will be shown in the following protocols that acid fuchsin does not enter the substance of the spinal cord and brain when it is injected into a lymph sac of a cardiectomized frog whose anterior lymph hearts have been destroyed. It may also be stated here that in very large cardiectomized frogs (225 to 250 grams) it is possible by injecting an acid fuchsin solution directly into the pulsating anterior lymph hearts to induce convulsions and to obtain ocular proof of the presence of the dye-stuff in the spinal cord and brain.

Experiments are now in progress on cardiectomized frogs in which I am attempting to cut off the connection between the anterior lymph hearts and the vertebral veins without in any way interfering with their pulsations. These experiments will, I hope, throw light on the question as to whether there is more than one backward route from the truncal lymph sacs to the central nervous system.

The following description of easily made experiments contains the proof of the assertion that for the success of Meltzer's experiments pulsating anterior lymph hearts are indispensable.

1. Two frogs of nearly equal weight are cardiectomized by the method of ligature. A strip of skin about a centimeter wide is removed from the back over the scapular portion of the pectoral girdle.

The anterior lymph hearts of both frogs are then exposed to view by removing the dorsal fasciae, a part of the dorsal muscles and the supra scapula. Care should be taken not to injure the lymph hearts during this operation. When it is seen that the exposed lymph hearts are pulsating well in both frogs and that no blood stained lymph is escaping from the field of operation, the lymph hearts of the one frog are quickly destroyed by means of a red hot file. Each frog now receives into the submaxillary lymph sac a fairly large dose of acid fuchsin ($1/10$ – $1/5$ mg. per gram of body weight). With a little practice the operations of cardiectomy, of destruction of the anterior lymph hearts and the injection of the solution can be made in about ten or twelve minutes in the case of a single frog.

The results of the above experiment are striking. The frog whose anterior lymph hearts are intact and pulsating well responds to the injection with convulsions, the usual sequence of symptoms being observed, while the frog whose lymph hearts have been destroyed retains a certain amount of irritability for four or five minutes or a little longer as the case may be and then dies. No sign of life can be obtained, the frog lies prone and gives no response when stimulated while the other has the typical extensor tetanus that follows the administration of acid fuchsin in the cardiectomized frog. In about thirty minutes after the destruction of the lymph hearts in the one frog as described, the spinal cord and brain of both frogs are removed and tested for acid fuchsin. The dyestuff is found only in the spinal cord and brain of the frog whose anterior lymph hearts are intact. Only in this frog does the dyestuff gain access to the interior of the skull and spinal column, within the time limits here considered. It is noteworthy, however, that in the case of the frog whose lymph hearts were destroyed the acid fuchsin is found in considerable amount on the outside of the spinal column along its entire length. The anterior or abdominal surfaces of the vertebrae

for example, take on a deep red color when touched with hydrochloric acid, thus showing that the fuchsin solution easily finds its way from the submaxillary into the prevertebral lymph sac.

Injections of solutions of strychnine or of morphine in the proper dose give the same results as above described; when the anterior lymph hearts are destroyed these drugs also fail to induce convulsions in cardiectomized frogs.

2. The same results are obtained when both of the cardiectomized frogs, the one with lymph hearts destroyed the other with them intact, are treated as follows. Both are placed in the vertical position but with the head down, so that the solution of acid fuchsin morphine, or strychnine may be retained in the extreme upper end of the cranio-dorsal lymph sac, which in the head down position of the frog forms a convenient pocket for the retention of the solution. The upper part of the cranio-dorsal sac communicates freely, via intervening lymph spaces with the anterior lymph hearts and it is therefore not surprising that the frog with intact lymph hearts soon falls into convulsions.

On account of the ease with which solutions are carried from the region of the head and the anterior part of the trunk to the anterior lymph hearts the experiment succeeds equally well when the head is immersed in a 1 per cent solution of acid fuchsin or of nitrate of strychnine after the skin has been removed from between the eyeballs and over the submaxillary lymph sac. In such an experiment the frog with the anterior lymph hearts intact falls into convulsions while its fellow shows no symptoms.

It is of interest to note that contrary to this experiment which shows rapid absorption from the exposed, upper part of the cranio-dorsal and submaxillary sinuses, we meet with entirely negative results when a cardiectomized frog, with all four lymph hearts intact, has his skinned legs immersed in a 1 per cent solution of acid fuchsin or of nitrate of strychnine. Indeed, a skinned frog may be immersed in one of these solutions for two hours, from his toes to a point that is well above the location of the posterior lymph hearts, without giving the least evidence of hyperexcitability or of convulsions during this time. This negative result is readily understood when it is recalled that the posterior lymph

hearts throw their contents into the transverse iliac vein which empties into a femoral vein and sometimes into the common iliac vein, and which, therefore, stand in no such relation to the membranes of the spinal cord and brain as has been shown to obtain for the anterior lymph hearts.

3. Similar results are obtained even when the injections are made into the prevertebral lymph sinus—that large lymph reservoir which lies immediately ventral to the spinal column and extends from the first or second vertebra to the lower end of the body cavity. To enter this sinus the injection needle should be passed into the basilar sinus in the roof of the mouth between the eyeballs, and then forced downward until the cervical part of the prevertebral sinus is reached, care being taken to keep the needle close to the ventral surface of the vertebrae. In this case also convulsions are seen only in the cardiectomized frog whose lymph hearts have been destroyed.

COMMENTS ON THE WORK OF EARLIER INVESTIGATORS WHO STUDIED THE ACTION OF DRUGS IN CARDIECTOMIZED FROGS

It appears that ligation or excision was not an uncommon procedure in the work of earlier investigators when they were studying the action of drugs on frogs. Dr. Reid Hunt has called my attention to the fact that this experiment was performed by Kölliker,²² Meyer²³ and Durdufi.²⁴

Occasional use of the procedure was made by Kölliker in his study of the action of hydrocyanic acid to prevent absorption and it may be noted that he expresses a doubt in one instance of the efficacy of the procedure in entirely preventing absorption, and Meyer's work also shows that use of the method was made only incidentally in his study of the action of hydrocyanic acid and calls for no further comment here.

Durdufi's researches on the point under consideration were also not extensive but they deserve a more extended notice. This

²² Virchow's Archiv, Bd. x, 283, 1856.

²³ Archiv für physiolog. Heilkunde, Bd. ii, s. 249, 1843.

²⁴ Arch. f. exp. Pathol. und Pharmakol., Bd. xxv, s. 447, 1889.

investigator, in the course of a research on the "pharmacological physiology" of the frog's heart made a few experiments on the absorption of drugs in frogs with heart standstill induced by muscarine, by helleborein or by ligature of the organ itself. He observed that when he injected a solution of atropine under the skin of the thigh of a frog whose heart had been brought to a standstill with muscarine, the heart was restored to activity in the course of a few minutes. The subcutaneous injection of 1/10 mg. of strychnine into frogs with the heart in muscarine standstill brought on the characteristic tetanus in a short time. When, however, the heart was put into the condition of systolic standstill by means of helleborein, or when it was cut out of the circulation by means of ligatures, then strychnine in the dose as before (1/10 mg.) no longer caused convulsions. From these experiments Durdufi concluded that in frogs with non-beating hearts absorption occurs only in the case of a *diastolic* cardiac standstill and not in cases of *systolic* standstill as induced by helleborein or by ligature of the heart. Nowhere is there any mention of the action of the lymph hearts though the author may have had this in mind in the muscarine-atropine experiment as he remarks that absorption and distribution take place in this case because the heart in muscarine standstill can give passage to fluids from the venous to the arterial side, this passage way being closed after ligature or helleborein standstill. We see, however, that Durdufi was quite mistaken in assuming that there can be no absorption and distribution of drugs in cardiac-tomized frogs.

Of equal or greater interest are the extensive researches of Ringer²⁵ and of Ringer and Murrell²⁶ on the action of potash salts and of aconitine on frogs with arrested circulation. These investigators, like Durdufi, Joseph and Meltzer and others, failed to note the work of the lymph hearts in carrying drugs to the spinal cord and brain, but ascribed the effects noted by them entirely to diffusion through the tissues. In regard to the action

²⁵ Journ. of Physiol., i, p. 86, 1878-79.

²⁶ Ibid., p. 239.

of aconitine on cardiectomized frogs Ringer and Murrell speak as follows:

To test whether aconitia can diffuse itself through the tissues irrespective of the circulation, we performed the following experiments on three frogs. We made a small incision through the thorax and cut the heart in half (subsequently verifying the operation by a post mortem), this of course completely arresting the circulation. Into two frogs, weighing respectively 23 and 35 grammes, we injected $1/25$ grain of aconitia (solution 1 in 100) under the skin of the back; the third we left unpoisoned to serve as a test frog.

The poisoned frogs completely lost sensation in two minutes, and in twenty-nine and twenty-four minutes respectively paralysis was complete, whilst in the unpoisoned frog, at the end of an hour sensation still continued in every part of the animal and voluntary power was good though somewhat weakened. Now these experiments clearly show how readily aconitia can diffuse itself and reach the central nervous system, without the help of the circulation.

Ringer's experiments on the action of potash salts on cardiectomized frogs are quite exhaustive and preceded those made with aconitine. As a result of numerous experiments, the results of which are arranged in tabular form, Ringer concludes as follows:

These figures themselves are sufficient to show that chloride of potassium can diffuse itself and paralyze the muscles without passing through the circulation, for in these experiments the heart being extirpated and the circulation therefore completely abolished, yet the muscles become much sooner paralyzed than the muscles of frogs subjected to mechanical arrest of the circulation, without poisoning.

In Ringer's experiments, after injection of the potash solution under the skin of the back, muscular irritability ceased in the anterior extremities in twenty-one hours, in the thighs in twenty-four hours, in the lower legs in forty-one hours, and Ringer is quite right in referring this slow action of the drug in cardiectomized frogs to diffusion and inhibition. We saw that while a *solution* of acid fuchsin is carried to considerable distances in communicating lymph sacs, yet the acid fuchsin itself (the *solute*) can only penetrate the muscles by diffusion, a process which is so slow that the

interior of the muscle requires hours to be reached, a fact which is well illustrated in Ringer's experiments. Had Ringer made use of a dyestuff he would undoubtedly have made a distinction between the primary, rapid, though very incomplete distribution of the potash solution and the subsequent slow passage of the drug into the tissues, and he would perhaps have hesitated to ascribe to diffusion the quick transfer of aconitine from the dorsal lymph sac to the cells of the spinal cord and brain.

The results of the experiments described by me in this paper must make it evident that Ringer's experiment with aconitine is but another example of the rapidity with which the surviving, pulsating anterior lymph hearts of the cardiectomized frog can carry a drug from a truncal lymph sac to the brain.

The observed action of the anterior lymph hearts in carrying on a low degree of circulation in the central nervous system for some time after cardiectomy would seem to be worthy of further study from several points of view. The influence of temperature and other conditions on the irritability of the lymph hearts are subjects which deserve attention. Ringer remarks that "early loss of sensation occurs after mechanical arrest of the circulation in the summer months, but not in winter," without venturing upon an explanation of the difference.

CONCLUSIONS

1. A fairly rapid distribution of solutions by means of non-vital agencies, such as capillarity, hydrostatic pressure (distension of lymph sacs, weight of the frog's body resting on the sacs) and diffusion, takes place both in dead and in recently cardiectomized frogs. The routes for this distribution are primarily the communicating series of lymph sacs, lymph sinuses and channels. The "tissue spaces" (Gewebespalten) of Meltzer can not serve as the paths for this rapid distribution as the resistance to motion in these minute spaces due to viscosity is so great that the effect produced by the driving force derived from surface energy must be practically nil. Progress in the "tissue spaces" can only be made by diffusion of the solute, a slow process, whose

action can be traced by the help of acid fuchsin and which is well illustrated in Ringer's experiments with salts of potash. The distribution of a drug in cardiectomized frogs, within the time limits with which we are here concerned (one-fourth to two hours), is not general, but very partial and limited and it depends upon the location and connections of the lymph sac selected for the injection, the amount of the solution injected, the tension of the lymph sacs, the state of the lymph hearts and other factors.

2. The appearance of convulsions in the experiments of Meltzer and his pupils, with acid fuchsin, morphine and strychnine depends entirely on the integrity of the anterior lymph hearts. If these organs have ceased to pulsate or if they have been excised or destroyed, convulsions will not be seen after the injection of one of the above-named drugs into cardiectomized frogs. The surviving, actively-beating, anterior lymph hearts keep up a certain degree of circulation for an hour or more, in the brain and spinal cord of the cardiectomized frog, thus making it possible for these organs to retain their responsiveness to stimuli. It is also the anterior lymph hearts that so quickly transport solutions of drugs from both the subcutaneous and the deeply situated truncal lymph sacs of the cardiectomized frog to the brain and spinal cord.

3. When the minimal effective dose of acid fuchsin, $1/40$ mg. per gram of body weight (Joseph and Meltzer), is injected into the cranio-dorsal lymph sac of a cardiectomized frog, a fractional part of the solution is driven into the cavity of the skull and of the spinal column by the surviving lymph hearts. Because the cardiac circulation has been eliminated, the drug can not be carried away to other tissues and hence the brain and spinal cord are free to take up and retain an effective quantity of the drug. In the intact frog, on the other hand, a quantity of the drug which suffices to induce convulsions in the cardiectomized animal is so thoroughly distributed throughout the entire body and so largely bound to other tissues which have a greater avidity for it, that not enough remains at the disposal of the brain and cord to affect those organs and to give a color reaction upon the addition of an acid.

4. Convulsions never occur after acid fuchsin unless the spinal cord and brain have absorbed at least enough of the drug to give a definite and unmistakable pink color on the addition of hydrochloric acid to the crushed tissue.²⁷ In all of the experiments of Joseph and Meltzer with cardiectomized frogs the brain and spinal cord always received that amount of the dye stuff which just suffices to induce convulsions, and to give the pink color with hydrochloric acid. That these authors were able to attain their results with such *small* quantities of acid fuchsin is due to the action of the surviving lymph hearts and to the increased sensitiveness of the spinal cord and brain toward acid fuchsin which results from the loss of the blood-circulation. This last factor is demonstrated by cardiectomizing at a time when the drug is already distributed throughout the body and when the spinal cord and brain already contain the minimum effective quantity for these organs. Under these conditions cardiectomy is quickly followed by convulsions, while the intact frog whose spinal cord and brain hold an equal quantity of the dye stuff has no convulsions. Cardiectomy, like the removal of the anterior third of the brain, causes a small quantity of the dye stuff in the central nervous system, that is, the minimum quantity that gives a pink color with hydrochloric acid, to manifest itself by convulsions.

5. It is therefore apparent that it is unnecessary to assume the existence of a special neutralizing or antitoxic substance (Meltzer) which circulates in the blood and ordinarily prevents convulsions, but whose absence in the cardiectomized frog enables acid fuchsin to induce convulsions in such small doses as 1/40 mg. per gram of body weight (Meltzer).

6. What has been shown to be true for the distribution of acid fuchsin in cardiectomized frogs holds true also, though with certain limitations, for morphine, strychnine and other drugs. Here also (morphine) it is unnecessary to assume the existence of a special antitoxic substance in the normal blood to explain the anomalous toxicity of the drug in the cardiectomized frog.

²⁷ Experiments are now in progress to determine the amount of this dye stuff in the brain and spinal cord of "normal" frogs at the time of the "late tetanus" (Barbour and Abel).

THE ACTION OF CAFFEIN ON THE MAMMALIAN HEART

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The action of caffein on the mammalian heart has been described by numerous investigators, employing various methods: with the circulation intact, with the cardio-plethysmograph, myocardiograph, by the artificial perfusion of the excised organ, etc. The results on the excised heart alone seem to be fairly uniform. With the other methods the reported results show less agreement. It seems to be conceded that small doses increase cardiac efficiency and larger doses have the opposite effect. However the border line between the beneficial and the harmful dosage has not been determined with any degree of accuracy. At Dr. Sollmann's suggestion this investigation was undertaken with the hope of clearing up the doubtful points.

The present study was directed mainly to the changes in the heart volume, including the individual excursions and tone; the blood pressure, heart rate and irregularities were noted incidentally and will also be briefly described, since these phenomena are all interrelated.

Methods. Fourteen experiments were performed with the cardio-plethysmograph similar to the type described by Y. Henderson (1). The ventricular volume only was recorded, the auricles being excluded from the plethysmograph. Recording tambours of 9 cm. diameter gave better details in the tracings, but with the larger quantities of caffein, the great increase in cardiac volume put the contained air under excessive tension, hence when the total volume changes were desired larger recorders (12 cm.) were used.

Eleven experiments were made with the early model of Cushny's myocardiograph (2). In this apparatus two upright rods are fastened to opposite sides of the ventricle and transmit the ventricular movements to a horizontal lever writing on a revolving drum. Five experiments were made with the right ventricle and six with the left ventricle.

Dogs were used exclusively, those not over 10 kg. in weight were found to be the most satisfactory. Morphin-ether anesthesia was employed in all but three experiments, in which morphin and Grehant's chloroform-alcohol anesthetic was given; under the latter mixture the animals were much quieter but the blood pressure was uniformly low. The results with the two anesthetics were similar. Curare was used when necessary. Uniform artificial respiration was administered by a motor driven bellows.

After laying bare the sternum, the vessels were cauterized and the heart was exposed by sawing through the mid-sternal line; further hemorrhage was arrested by the cautery. The cautery was found to be more satisfactory than the ligation method.

A 1 per cent solution of the pure caffein base dissolved in normal saline solution was injected into the femoral vein from a burette. The dosage is always stated as milligrams of caffein per kilogram of body weight. The injections were usually made rapidly, within five to fifteen seconds with small doses, but somewhat more slowly as a rule with larger doses. A number of slow injections were also made. With the large doses of caffein considerable saline solution was necessarily injected and suitable control experiments were made by injecting like quantities of pure saline solution.

Effect on blood pressure. The action of caffein on the blood pressure, heart rate and irregularities has been described in detail in a previous paper (3) and will need only a brief recapitulation. The initial blood pressure averaged considerably lower in the present series, on account of the severity of the operation, but the response to caffein was essentially the same (4). Following the rapid intravenous injection of caffein, there is a brief preliminary rise in blood pressure (5 to 10 mm.) of about five seconds

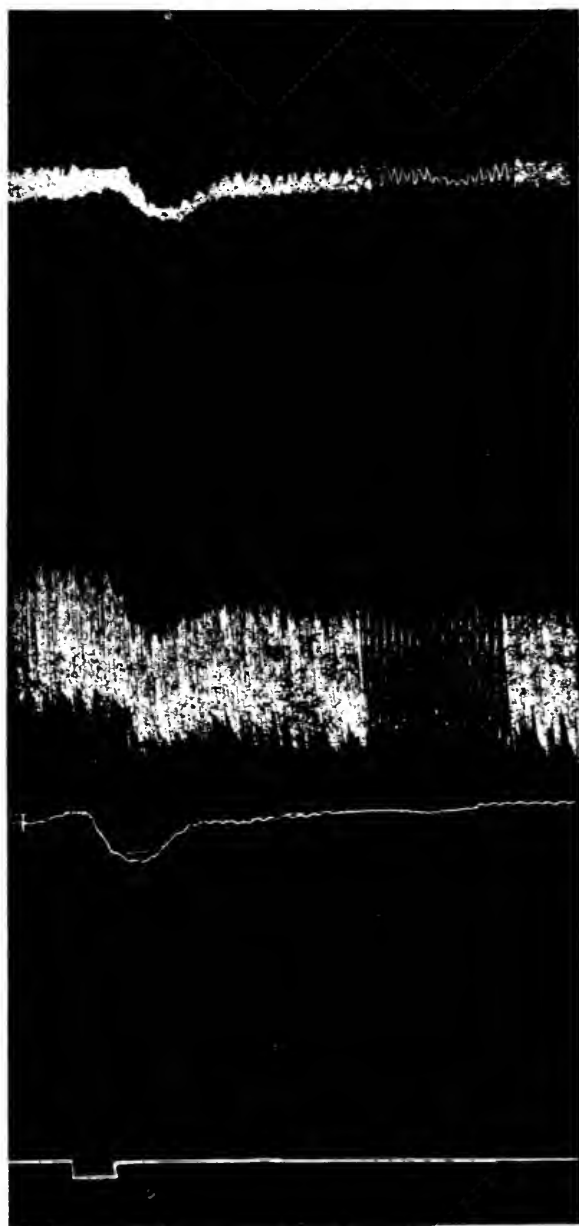


Fig. 1. ACUTE CHANGE IN CARDIAC VOLUME: DECREASED VOLUME WITH SMALL DOSES OF CAFFEIN—CARDIOPLETHYSMOGRAM

Upper curve, membrane manometer; Middle curve, volume curve of heart; Lower curve, blood pressure—mercury; Base line, zero blood pressure. Caffein injected at the signal (3 mg. per kilogram; previous dose, 2 mg. per kilogram). The speed of the drum is increased temporarily toward the right side of the tracing.

duration. This is followed by an abrupt fall which is completed in about fifteen seconds. This acute fall in blood pressure increases progressively with successive doses to an average maximum fall of about 30 mm., when 40 to 60 mg. of caffein have been injected, with further increase of dosage as the permanent level of the blood pressure is progressively lowered, the fall diminishes in extent. The acute fall is of short duration. With the smaller doses of caffein, it is usually succeeded by a slight rise.

Level of blood pressure with successive doses. With a total quantity of caffein 10 mg. fourteen of twenty-two uncomplicated experiments gave an average rise in blood pressure of 12 to 15 mm. above the original level; the others a very slight rise (5 mm.) or none at all. In five experiments, the original blood pressure was below 60 mm.; in four of these, the caffein rise did not exceed 10 mm., in the fifth the rise was from 62 to 110 mm.

With the next dose of 10 mg. (total 20 mg.), the average blood pressure fell slightly but remained above the normal until a total of 40 mg. had been injected. When the dosage exceeds 40 mg. the pressure falls more or less rapidly until death, but a constant low level may be maintained for some time (4).

Acute changes in heart volume. It was shown in our previous study that the intravenous injection of caffein is followed by two sets of changes, which must be considered separately: namely, first, those which occur acutely and which pass off promptly—the “acute changes” and second, the “permanent” changes, which set in after the acute effects, but which are lasting and cumulative.

The acute changes will be discussed first. These are characterized by a slight primary rise of blood pressure, which is really due to the saline injection, and by the sharp fall of pressure, which is produced specifically by the caffein when it is injected rather rapidly into a vein. The corresponding volume changes in the heart are demonstrated most clearly by the cardio-plethysmograph and are essentially as follows:

Behavior of the cardio-plethysmograph during the injection rise. With the primary rise in blood pressure accompanying the in-

jection of caffein, there is usually a slight increase in cardiac volume, returning to normal as the pressure falls. With large doses of caffein, this increase in volume is greater and merges into the more marked increase in volume coincident with the

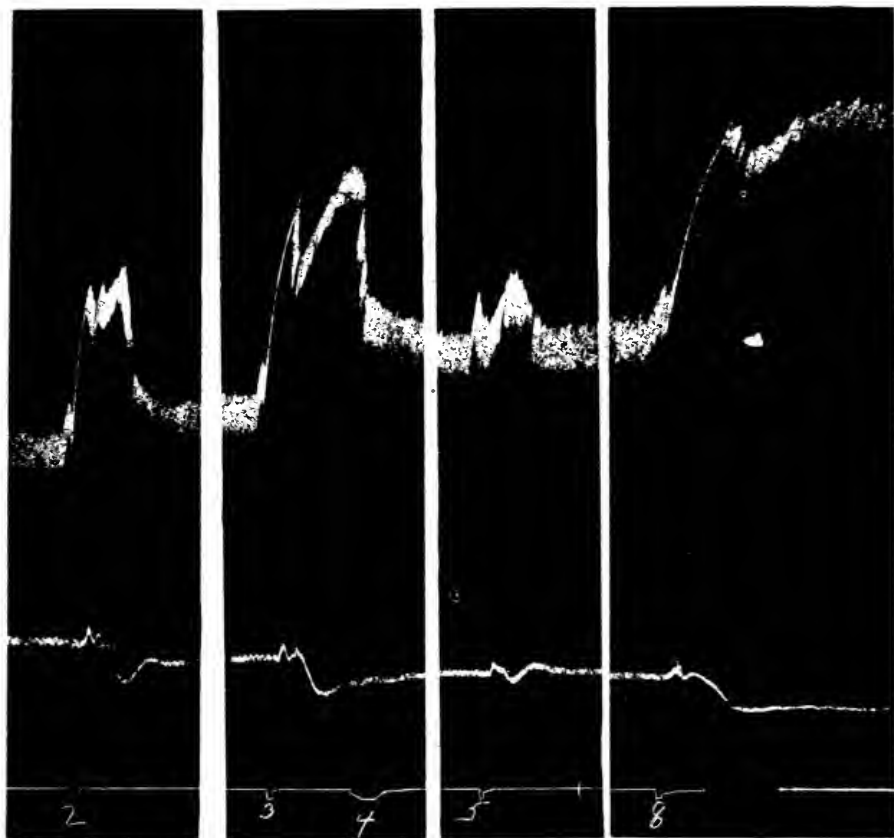


FIG. 2. ACUTE CHANGES IN CARDIAC VOLUME—CARDIAC PLETHYSMOGRAPH

Increase in volume with large doses of caffein (20 mg. per injection; total quantity injected; 100 mg. at 2; 120 mg. at 3; 140 mg. at 8). At 5 normal saline equivalent to the saline in 20 mg. caffein solution was injected. The signal 4 indicates the space of thirty seconds.

fall in blood pressure (fig. 2). The greater volume of fluid entering the heart subsequent to the injection would seem to be the correct explanation of the increase in volume at this period

Behavior of the cardio-plethysmograph during the injection fall of blood pressure. Accompanying the fall in blood pressure there is usually a change in cardiac volume. With *small doses* of caffeine the change consists usually in a decrease in volume which is generally comparatively small, but exceptionally may be considerable.

With increase in dosage the volume variations become more pronounced and with *large doses*, generally with 20 to 40 mg., the volume is *increased*, the increase becomes progressively greater with the dosage; finally the heart stops in an extremely distended position (fig. 2).

Statistical data. With small doses of caffeine (2, 3 and 5 mg. per kilogram) the volume of the ventricle usually decreased, namely in thirty-three of forty-one injections, and was increased five and unchanged three times. These exceptions are not of sufficient importance to merit discussion. With the next dose of 10 mg. (total 20 mg.) the results are less uniform, the volume being increased, decreased and unchanged in an equal number of injections. This quantity (20 mg.) seems to be about the limiting point between the doses of caffeine which cause decrease and those causing an increase in cardiac volume, during the acute fall in blood pressure. With the next injection of 20 mg. (total 40 mg.), the volume increased in eight of twelve injections, and decreased in four. With still larger quantities of caffeine the volume increased in thirty-one of forty-three injections, and decreased in twelve. These exceptional decreases in volume were confined to three experiments which were anomalous throughout. In two of these the volume increased with a total quantity of caffeine of 110 and 140 mg. It may be that the hearts were dilated at the beginning of the experiment and caffeine reduced their size as in one myocardiograph experiment (206) to be discussed later. In the third experiment (195) with 40 mg. and above, a marked decrease in volume preceded the dilatation. Other conditions were not abnormal in these exceptional experiments.

With a very low blood pressure after large quantities of caffeine, the injections may result in a rise of blood pressure, in which case, however, there is the usual accompanying increase in volume, which may precede or be synchronous with the rise (experiment 204, 205).

Myocardiograph experiments in the acute fall in blood pressure. The myocardiograms are better adapted for studying the varia-

tions in the systolic and diastolic excursions; but the volume variations can also be estimated by representing the volume as a line drawn midway between the systolic and diastolic base lines. The results agree fairly uniformly with the plethysmograms, although they give a somewhat smaller number of decreased volume records with the smaller quantities of caffein. With the larger doses the results are more uniform with the myocardiograph, as there is but one experiment in which the volume decreased after 40 mg; in experiment 206 (right ventricle) the volume decreased until a total of 60 mg. was given.

These results agree closely with Aubert's (5) observations (made by inspection only) on the intravenous injection of caffein into rabbits. With 30 mg. of caffein, he observed a primary decrease of the cardiac volume and excursions, followed by relaxation and distention, especially of the right ventricle. With 100 mg. there was a brief period of asystole followed by cardiac relaxation and distention as before. With larger doses the heart stopped in the extremely distended position.

The relation of the cardiac changes to the acute fall of blood pressure. In our previous paper, we concluded that the acute fall of blood pressure must be due essentially to acute cardiac depression. The direct observation of the cardiac volume shows that there is such a relation, the changes in volume coincide in time, or somewhat precede the blood pressure fall. The amplitude of the cardiac excursion is also decreased during the fall. However, the relation is apparently not a quantitative one; for while the heart continues to dilate and the excursions progressively decrease as the total dosage of caffein increases, until death occurs, the acute blood pressure fall becomes less and less, as the permanent level of blood pressure is lowered (fig. 2). This means simply that other factors enter into play, namely, that the blood pressure ceases to reflect the cardiac changes when the whole circulation is altered by the permanent vasodilation and cardiac distention, and the consequent reversal in the distribution of the blood, which characterizes the larger doses of caffein.

Time relations of the blood pressure fall and cardiac changes. The volume changes and blood pressure changes are almost synchronous. Both occurred promptly with rapid injections, and slowly or imperfectly with slow injections. The tracings often show no perceptible difference in the time of the two sets of changes; but when a difference is perceptible (in four of ten experiments), the cardiac changes always start and reach their maximum a trifle before the blood pressure. In experiment 191, with 100 to 140 mg. doses, the heart was quite visibly dilated before the blood pressure began to fall.

The relation between the extent of the acute volume and blood pressure changes. With small quantities of caffein there seems to be no relation between the variations in volume and the degree of the fall in blood pressure.

There are nine experiments showing a decreased or unaffected volume with each injection of 2, 3 and 5 mg. of caffein. In all of these the fall in blood pressure increases progressively with the dose but the volume variations are by no means regular; in three experiments (180, 3, 8) the decrease in volume varied inversely to the fall in pressure; in three others (184, 190, 191) the volume varied directly (not mathematically however) with the fall in blood pressure, and in two experiments, with the usual blood pressure changes, the volume changes were quite irregular.

At a somewhat variable point, usually about 20 to 40 mg. but occasionally with larger quantities, the acute dilatation increases progressively with each dose until death, in the majority of experiments, while the acute fall of blood pressure reaches its maximum before half the fatal dose is reached (with 40 to 60 mg. in eight of ten experiments). The blood pressure in the plethysmographic experiments averages 95 mm. at the time when the acute fall of blood pressure has reached its maximum. As was mentioned above, after large quantities of caffein occasionally the blood pressure is maintained for some time at a very low level (30 mm.) and the injection frequently resulted in a rise of blood pressure but the heart dilated as usual with the large doses.

The cardiac excursions during the acute fall in blood pressure; with small doses of caffein (to 10 mg.). There was little variation in the amplitude of the excursions during the fall in blood

pressure; the excursions usually remained unchanged or were slightly lessened, or sometimes slightly increased. There seems to be no relation between the variations in amplitude and the extent of the fall in blood pressure. There seems to be some connection between the change in volume and the amplitude of the excursion; the greater diminution in excursion was in those curves with the largest volume variations, but this also is uncertain.

Of forty injections with the plethysmograph there was no variation in amplitude in twenty-one and a decrease in nineteen. Of twenty-six injections with the myocardiograph, there was no variation in three, a decrease in fourteen and an increase in nine.

With larger quantities of caffein (above 10 mg.) there is a diminution in excursion amounting to a maximum of 30 to 50 per cent.

The decrease in excursion is roughly proportional to the increase in volume. With the sharp marked increase in volume with a large total quantity of caffein the excursions at times become very greatly decreased, the heart being practically in a condition of asystole. With the larger quantities of caffein the excursions may be very irregular in rate and amplitude.

Santesson (6) with the plethysmograph, described diminished excursions with small doses during the fall and increased excursions in the recovery. I observed this secondary increase of the excursions with the myocardiograph but not with the plethysmograph.

Changes in the systolic and diastolic tone during the acute fall in blood pressure. The usual slight decrease in cardiac volume which was produced by small doses (to 10 mg.) was dependent upon an increased systolic excursion and a diminished diastolic relaxation (fig.1). With the next dose of 10 mg. (20 mg. in all), the curves were irregular, with a general tendency toward lessening of the systolic excursion, with the diastolic excursion unaffected or somewhat increased. As the volume becomes more markedly increased, the characteristic result is the great decrease in the systolic excursion with a relatively smaller increase in the dias-

toxic excursion; this results practically in a condition of asystole when the lethal point is approached.

Permanent cardiac changes produced by the smaller doses of caffeine. The persistent effects of caffeine on the blood pressure



FIG. 3. MYOCARDIOGRAM—LEFT VENTRICLE

Decrease in amplitude with large doses during fall of blood pressure and cardiac dilation—Caffeine 20 mg. injected at the first signal (total dose 80 mg). Upper curve, myocardiogram; Lower curve, blood pressure; base line, zero blood pressure.

described on page 81, of our previous paper (3) were explained as follows: "*constant and pronounced vasodilation, with varying degree of cardiac stimulation if the total dosage of caffein is small, or cardiac depression if the total dosage is large.*" These explanations were deduced particularly from the oncometric changes.

The direct cardiac studies of the present series show changes in volume and excursion corresponding with the blood pressure changes. Following an intravenous injection, when the blood pressure has reached its permanent level the conditions of the heart are as follows: After the small doses of caffein (to 20 mg.), when the blood pressure is normal or slightly raised, the cardiac volume generally remains somewhat decreased, with the systolic excursion increased and the diastole relaxation lessened, i. e., the cardiac tone is raised (fig. 1); the amplitude of the excursions is generally unchanged but may be increased; the heart rate is somewhat increased.

The cardiac volume. As the blood pressure recovers from the acute injection fall, the volume variations are usually also affected. With a total quantity of caffein 20 mg. in about an equal number of injections the volume remained decreased (twenty-two) or returned to the preinjection volume (twenty-one) but less frequently was increased (nine). The volume changes were about equally divided among the different doses (2, 3, 5, 10 mg.). With the myocardiograph with the same dosage, the volume of the left ventricle was decreased fifteen, unchanged five and increased four times; the volume of the right ventricle was decreased and unaffected in an equal number of injections (eight).

The cardiac excursions. With the two methods of experimentation the results were not uniform: the *plethysmograms* show little variation in excursion with the smaller quantities (20 mg), but exceptionally the amplitude may be slightly augmented or diminished. In one experiment only (187) was the amplitude constantly increased with this dosage, and in one experiment (181) the amplitude seemed to be augmented although the excursions were very small.

Myocardiograph. With this recorder, the excursions were usually augmented and the results with the two ventricles were

fairly uniform. The excursions of the right ventricle were increased nine times and unaffected twice; of the left increased twelve, unchanged four and lessened six times.

The heart rate. Small doses (20 mg.) resulted in a somewhat quickened heart rate as has been abundantly substantiated by earlier investigators. The occasional decrease in rate dependent upon primary vagus stimulation was not observed in this series.

Cushny and Van Naten (2), employing the myocardiograph on the right auricles and ventricles of dogs, found caffeine 0.10 mg. to be without effect other than acceleration of the rate (this dose would correspond to about 10 mgr. per kilogram). With larger quantities the auricular systole was lessened; then the ventricular diastole was decreased and finally the ventricular systole was diminished; no increase in the systolic excursion was mentioned. Still larger doses (1 gram) resulted in arrhythmia and finally the heart stopped in extreme distention. (The doses here given refer to the total dosage not to grams per kilogram of animal weight.)

Permanent cardiac changes produced by the larger doses of caffeine (40 to 100 mg.). These cause a progressive fall in blood pressure and with this dosage the acute fall is accompanied by cardiac dilatation and the recovery from this dilatation is less and less complete, i. e., the tone of the heart is permanently and progressively lowered (fig. 2). The excursions are also reduced, especially the systolic contraction; the heart rate usually increases progressively but may be slowed; irregularities are frequent.

The cardiac volume. The volume changes are somewhat irregular with caffeine 40 mg. (in both the plethysmograph and myocardiograph experiments); with larger quantities however the volume usually increased and did not return to normal. The permanent increase in volume starts with a fairly uniform quantity of caffeine, usually from 40 to 60 mg. (table I) as the dosage is further increased, the dilation becomes more and more marked until a total of 100 to 140 mg. is reached. About this point there is a further more and more marked permanent increase in volume.

TABLE 1

Total quantity of caffein causing some degree of permanent cardiac dilation

	CAFFEIN MILLIGRAM PER KILOGRAM						
	10	40	60	80	100	140	160
Number of experiments with plethysmograph.....		4	3		1	1	
Number of experiments with myocardiograph							
Right ventricle.....		1		1	2		1
Left ventricle.....	1	3	2				

It is noticeable that much larger doses of caffein appear to be required to produce permanent dilatation of the right ventricle than of the left ventricle, but the number of experiments is not sufficient to warrant any conclusions.

There were two quite atypical experiments, one with the plethysmograph (181) and one with the myocardiograph (206). In these the volume decreased very considerably until caffein 100 and 140 mg. respectively had been received which dose initiated the progressive increase in volume. In the myocardiograph experiment, it was definitely noted that the heart was dilated at the beginning of the experiment and the volume was visibly diminished by caffein; presumably the condition was similar in the second experiment.

Two other experiments also required unusually large quantities of caffein to produce permanent dilation, but in these the volume remained practically at the original level and was not markedly decreased. In none of these exceptional experiments were other conditions unusual.

The cardiac excursions. In all but exceptional instances, the amplitude of the excursions was gradually lessened by the larger dose of caffein. In one experiment (206) in which the volume was markedly decreased until 100 mg. had been received, the amplitude was gradually increased from 10 to 28 mm. with caffein 40 mg. and was then gradually lessened. In experiment 198 the excursions were somewhat increased through caffein

80 mg. In both experiments the heart rate was increased. There were individual instances in which a lessened heart rate resulted in increased excursion.

The heart rate usually increases progressively with increase in dosage to a maximum of two hundred or more per minute; however, the maximum attained in this series was below that reported by other observers. In about one-third of the experiments the rate was somewhat increased when 40 to 60 mg. had been injected, beyond which it progressively decreased, so that rates of fifty to seventy per minute were not infrequent. In the latter experiments the blood pressure was usually low.

Cardiac irregularities produced by caffeine were not made the subject of especial study. They are usually initiated during the acute fall in blood pressure with quantities of caffeine that lessen cardiac tone (40 mg. and above); and usually disappear fairly promptly. As the dosage increases, the irregularities tend to become permanent. They affect both the rate and amplitude of the excursion. There were experiments without irregularities except during the acute fall in blood pressure.

Maximal doses of caffeine above 100 to 140 mg. The cardiac changes progress rapidly. As this dosage is approached, each injection of caffeine produces a more and more marked dilation, with less and less perfect recovery, so that the heart remains very much dilated. When the dosage reaches 100 to 140 mg. this dilation becomes very great and there is little tendency toward recovery, successive doses increase the volume and lessen the amplitude of the excursions and finally, shortly after an injection there is no recovery, the heart stopping in extreme diastole after more or less vermicular movement (fig. 2).

Individual variations in rate and rhythm become more marked but offer no further peculiarities worthy of note.

Discussion of the relation between the blood pressure, heart rate, amplitude of excursion and output. It was stated above that caffeine (up to 20 mg. per kilogram) usually causes an increase in blood pressure of a few millimeters, a moderate increase in heart rate and leaves the amplitude of the cardiac excursion unaffected or somewhat increased; larger quantities progres-

sively lower the blood pressure, increase the heart rate and decrease the excursion. These conditions were compared in nine plethysmograph experiments; in these with C. 20 mg. there was an average rise in blood pressure of about 15 mm. (from 70 mm. to 87 mm.) with an increase in heart rate of about thirty beats per minute, with the excursions practically unchanged; larger quantities caused the usual progressive lowering of blood pressure, increase in heart rate and decrease in amplitude. It would seem then that the rise in pressure in these experiments is due mainly to the increased heart rate. As the dosage is increased, the lessened excursions more than offset the increased rate, and the output per unit of time is therefore diminished and so the blood pressure falls. This result agrees with the generally accepted experimental results, that in spite of the increasing heart rate the blood pressure progressively falls when the caffein dosage becomes large. In the myocardial experiments the results were not so uniform and there were a few experiments in which other experimental procedures prevented this comparison. In four experiments with the usual blood pressure rise, the rate was very slightly increased but the amplitude of the excursions was increased; in others the rate was somewhat increased, but the amplitude was practically unchanged. It would seem then that with caffein a rise in blood pressure may result either from an increased excursion with heart rate not greatly modified or vice versa, with the excursion slightly modified but with the rate increased.

The vaso-dilation produced by caffein introduces another factor in this relation. This action, of course, tends to lessen the rise in blood pressure which would otherwise result from the increased heart rate or the augmented excursions. This vascular relaxation, although decreasing the blood pressure-raising power of caffein is probably of considerable value, as lessening the peripheral resistance and by increasing the blood flow through the part per unit of time.

CONCLUSIONS

1. *During the acute fall in blood pressure*, subsequent to a rapid intravenous injection of caffein. (a) Within a total quantity of caffein 10 mg. per kilogram the cardiac volume ("tone") and the amplitude of the excursion are usually unchanged. (b) With larger quantities, the volume progressively increases (or the "tone" decreases) and the amplitude of the excursions decreases.

2. *Permanent changes*. (a) Within a total quantity of caffein 20 mg. per kilogram, there is a moderate rise in blood pressure, an increased heart rate, a decrease in heart volume (or an increase in "tone"); there may or may not be an increase in the amplitude of the excursion. (b) With larger doses, the blood pressure progressively falls, the heart rate increases, the volume increases (or the "tone" is lessened), the amplitude of the excursions decreases.

It is a pleasure for me to acknowledge my indebtedness to Professor Sollmann for his aid in the experimentation and for revising the manuscript.

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THE ACTION OF GLANDULAR EXTRACTS UPON THE AMOUNT OF EPINEPHRIN IN THE BLOOD

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Fraenkel¹ has shown that the unstriped fiber of the uterus can be used to determine an excess of epinephrin in the blood of patients with Basedow's disease and chronic nephritis. He used blood serum. He found that strips of uterine tissue reacted to epinephrin in the dilution of 1 to 20,000,000. The serum of healthy persons usually acted in a dilution of 1 to 5 and 1 to 10, in a few cases in a dilution of 1 to 20 and 1 to 40, and three times in a dilution 1 to 50, but not beyond this dilution. He found the serum in Basedow's disease was active in a dilution of 1 to 400. He estimates the quantity of epinephrin in the blood to be about 12.5 mgs.

One² of us was the first to show that the adrenal extract relaxes the tonus and inhibits the rhythmic contractions of the intestine.

Magnus³ in 1905, showed that suprarenin, 1 to 20,000,000, inhibits the rhythmic contractions of the intestines.

Hoskins⁴ has shown that the rhythmic contractions of the intestine of the rabbit are inhibited by epinephrin, 1 to 400,000,000. We have shown that of the ductless glands there are none which give this peculiar reaction by the intestine to epinephrin.⁵

Cannon and de la Paz⁶ have shown that the adrenals can be excited by emotional impulses, and that the excess of epinephrin

¹ Archiv. für experiment. Pathol. und Pharmakol., Bd., lx, p. 394, 1909.

² Medical Bulletin, xix, p. 376, 1897.

³ Pfüger's Archiv., Bd., cviii, p. 48, 1905.

⁴ Jour. of Pharmacol. and Exper. Therapeutics, iii, no. 1, p. 93.

⁵ American Medicine, March, 1911.

⁶ Amer. Jour. of Physiol., xxviii, p. 64, April, 1911.

in the cat can be detected by the intestinal strip of the same animal. They obtained the blood from the vena cava above the opening of the adrenal veins, by a snip in the femoral vein, through which they passed a catheter. This blood was defibrinated and tested. The normal blood showed no epinephrin reaction, but excited the contractions and tonus of the intestine.

Cannon and Hoskins⁷ have also shown that in cats asphyxia and sciatic irritation increases the amount of epinephrin in the blood. They narcotized the animals with urethane and used the intestine of the rabbit for the test with defibrinated blood before and after asphyxia and sciatic irritation.

Ether, according to Elliot,⁸ causes a centrally excited loss of epinephrin in the medulla of the adrenal.

Delbet, Herrenschildt and Beauvy,⁹ found that chloroformization causes epinephrin to diminish and to completely disappear from the medulla of the adrenal, to reappear twelve hours after the use of the chloroform. Chloroform also causes considerable changes in the cortex of the adrenals. Kehrer and Fraenkel also noted that etherization materially diminishes the activity of uterine muscle as regards epinephrin.

Hoskins¹⁰ by feeding guinea pigs with dessicated thyroid and weighing the adrenals of their offspring found them depressed in weight by 53 per cent. In thyroidectomized guinea pigs he found that the adrenals of their offspring contained an average hyperplasia of 20 per cent. Normal animals fed on 5 to 15 mgs. of dessicated thyroid had an average hypertrophy in the adrenals of about 25 per cent. He holds that these results support the theory that thyroids normally stimulate the adrenals.

TESTS FOR EPINEPHRIN IN THE BLOOD

Our experiments were made upon cats deeply narcotized with urethane. The rabbits were also deeply narcotized with the same drug. The blood of the cat was obtained from the vena

⁷ Amer. Jour. of Physiol., xxix, p. 274, 1911.

⁸ Jour. of Physiol., xliii, no. 6, p. xxxii.

⁹ La Presse Médicale, no. 19, p. 200, March 6, 1912.

¹⁰ Jour. Amer. Med. Assoc., lv, no. 20, p. 1724, 1911.

cava according to the method of Cannon, and defibrinated. Then as a rule, the filtrate of 0.1296 grams of the dry powdered extract was injected per jugular. The filter was usually absorbent cotton although a paper filter was also used. After the usual wait of three and one-quarter minutes blood was again drawn from the vena cava, and defibrinated. With the albumens we waited from three and one-quarter to 30 minutes. Then a small segment of the intestine was obtained from the narcotized rabbit, attached by a pin hook to the bottom of the tube and by a pin and thread to Porter's heart lever. Then Ringer's solution was added, and the contractions noted; then normal blood until the contractions were uniform, when the blood, after the injection of the animal extract, was added. The saline and blood were drawn from the glass tube by means of an exit tube at its bottom, and oxygen was bubbling into the glass by means of an inlet tube at the base of the glass tube. In changing from normal blood to the blood affected by the animal extract the lever rises some. We have made over eighty experiments.

Thyroid, iodothyrim (fig. 1), parathyroid, thymus, infundibulin (fig. 2), pineal, pancreas, ovary and orchitic extract produced the epinephrin fall of tonus and a temporary inhibition of the rhythmic contractions. The thyroid, in causing an increase of epinephrin in the blood, supports Fraenkel's facts in Basedow's disease and also the results of Eppinger, Falta and Rudinger.

Spleen extract free of albumen was without effect.

The short duration of the epinephrin dip is partly due to the fact that all the above animal extracts except the adrenals, when injected, increase the tonus and often the extent of the rhythmic contraction and counteract in part the effect of the epinephrin.

Dr. Oswald Schwarz,¹¹ found that after the subcutaneous injection of a solution prepared from two fresh hypophyses of a horse into a rabbit the animal had paresis of the posterior extremities, then of the anterior extremities, marked dyspnoea, with evacuation of urine and feces. The animals died in twenty-four to thirty-six hours. When he made the test for the epinephrin

¹¹ Wien. Klin. Wochenschr., p. 984, 1909.

present by the rise of the blood pressure none was found in the adrenals. He found horse's pancreas, thyroid and adrenals gave the same result. Muscle and liver were considerably less toxic, but there was no epinephrin in the adrenals. The injection at intervals



FIG. 1. EFFECT OF IODOTHYRIN PER JUGULAR UPON AMOUNT OF EPINEPHRIN
IN THE BLOOD

N.B., normal blood; *I.B.*, iodothyron blood. Time, in four seconds.

of horse serum from one and one-half to two months in a rabbit, in order to obtain a precipitating serum, caused the epinephrin to be absent from the adrenals. He believes his experiments prove that foreign albumen is a poison, and when given in lethal doses

by subcutaneous injection influences in a very intense negative way the chromaffine system.

In the case of the thyroid, pineal, thymus, pancreas, ovary,

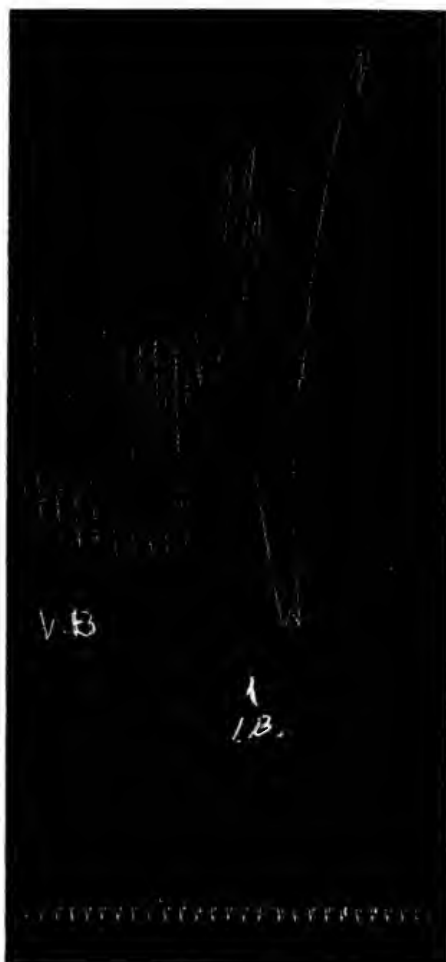


FIG. 2. EFFECT OF INFUNDIBULIN (20 PER CENT EXTRACT OF THE POSTERIOR PART OF THE PITUITARY) UPON THE AMOUNT OF EPINEPHRIN IN THE BLOOD

orchitic extract and parathyroid, we boiled them and their solution was filtered through paper whilst hot. Heller's nitric acid ring

test showed no albumen in the filtrate. In some cases we added 0.059 cc. of dilute acetic acid with a little sodium chloride, and then boiled the extract and filtered. The first filtrate was taken and contained no albumen when tested by Heller's ring test. Infundibulin does not contain albumen.

The antithyroid serum of Moebius (Merck) when given in doses of 0.177 cc. by the jugular causes an excess of epinephrin in the blood.

Diphtheria antitoxin made chiefly of globulins, in doses of 0.354 cc. by the jugular, caused an epinephrin effect upon the intestine.

Diphtheria antitoxin serum gave, in doses of 0.118 to 0.354cc. by jugular, a marked epinephrin reaction, which persisted for a half-hour afterwards.

We found that 0.118 cc. of egg albumen (fig. 3) by the jugular gave a marked epinephrin reaction. The same was true of peptones (fig. 4). The albumen of muscle extract and liver extract also gave an increase of epinephrin in the blood. Hence we can draw the conclusion that foreign albumens increase the amount of epinephrin in the blood. But our glandular extracts were free of albumen, as far as Heller's ring test goes. Iodothyryn and infundibulin being free of albumen, and nearer a state of chemical purity, we can infer that they stimulate the adrenals to increased activity. As to the other extracts we can not state whether it is due to a hormone or to some protein which causes an increase of epinephrin in the blood. We will have to wait until the chemists can produce the hormones in a state of purity. Some of these glands contain cholin, which increases the amount of epinephrin in the blood, according to recent experiments by us.

Pettit and Girard¹² found that, in a horse used in the preparation of antitoxin for diphtheria, the adrenals were in a state of general excessive secretory activity, especially marked at the level of the fasciculate layer and the medulla, with slight fatty degeneration.

Pettit and Girard¹² found in the horse who served many years

¹² Compt. rend. de la Soc. de Biol., p. 272, 1905.

in the production of antidiphtheritic and pest sera the hypophysis was the seat of a slight fatty degeneration with vascular congestion and an active proliferation of cells.

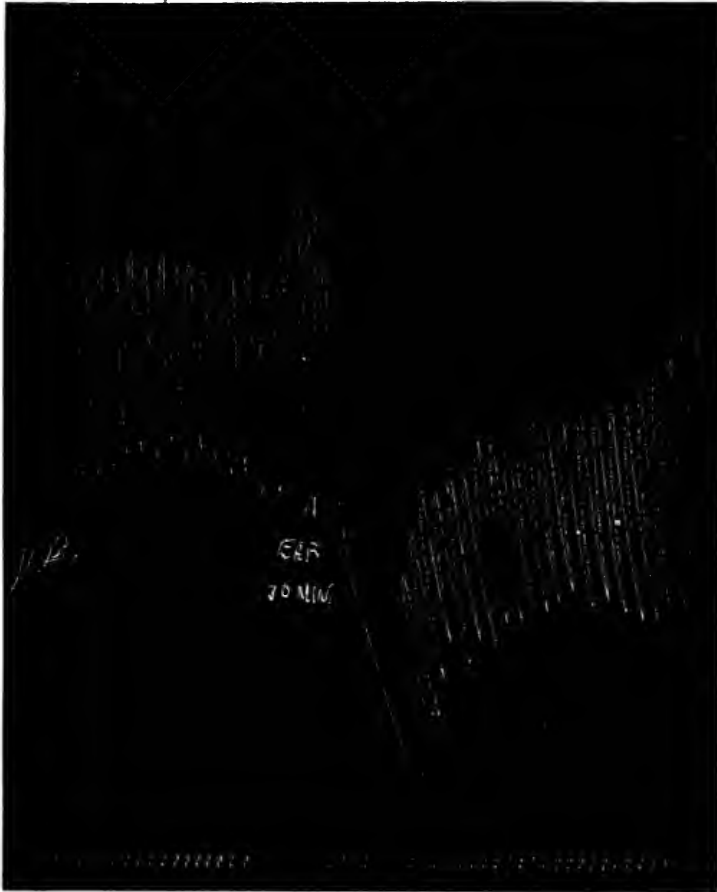


FIG. 3. EFFECT OF 0.118 C.C. OF EGG ALBUMEN UPON THE AMOUNT OF EPINEPHRIN IN THE BLOOD, THIRTY MINUTES AFTER THE INJECTION PER JUGULAR.

Perrier¹³ also confirms this statement as regards the horse's adrenals in the production of the antitoxin for diphtheria.

¹³ Perrier, Thèse pour le Doctorat en Médecine, p. 39, 1909.

That removal of a gland is followed by extensive changes in many other glands has been shown by Ascoli and Jegnani,¹⁴ who found, after removal of the hypophysis in dogs, changes in the

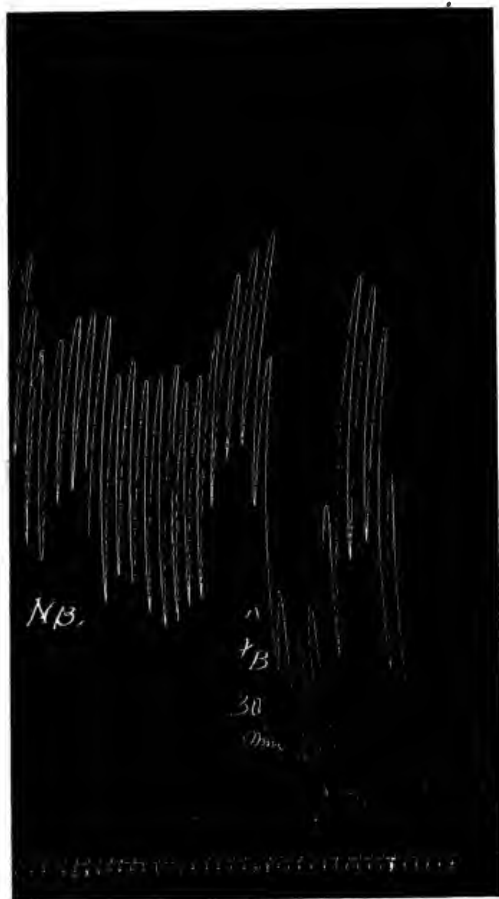


FIG. 4. EFFECT OF 0.0648 GRAM OF PEPTONE (MERCK) UPON THE CONTENT OF EPINEPHRIN IN THE BLOOD.

testicle, which did not develop. There were no spermatozoa, the ovary remained in the state of primitive follicles, the spleen was small, while no Malpighian bodies were seen, the thymus was only

¹⁴Münche. Med. Wochenschr., no. 10, p. 518, March 5, 1912.

one-fifth to one-sixth its normal weight, the lobes had disappeared, the tissue was loose, the lymphoid elements scarce, the separation between the cortex and medulla was lost, the concentric corpuscles were numerous and confluent. The thyroid had no marked microscopic changes, except it was small, atrophic and the epithelium was flattened. The adrenals contained hemorrhages. The cortex of the normal adrenal has the glomerular, fascicular and reticulated zones, whilst in the operated animal there was no differentiation of the two inner zones; they were as one, their cells enlarged and coarse, filled with drops of fat and lipoid bodies. Here are five glands affected by the removal of one.

The removal of the hypophysis causes changes in the formation of the lipoids of the adrenal cortex.

It is evident that the glands with an internal secretion are closely interlocked in function and that a diminution or an excess of activity in one is followed by anatomical and functional changes in several others.

That several glands increase a secretion was well illustrated in that of milk, where we have five—infundibulin, thymus, pineal, corpus luteum and mammary. Each one of these greatly augments the secretion of milk when a solution of them is injected per jugular in the goat.¹⁵

¹⁵ Ott and Scott, *Therapeutic Gazette*, October, 1911.

ON THE ACTION OF SODIUM CITRATE UPON MAMMALIA, WITH ESPECIAL REFERENCE TO ACQUIRED TOLERANCE AND TO ITS ACTION UPON THE CEREBELLUM

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1. THE TOXIC DOSE (SUBCUTANEOUS ADMINISTRATION)

The experiments which are about to be described were undertaken, in the first place, in order to ascertain whether it is possible to accustom an animal to citrates by repeated administrations, so that an otherwise highly toxic or even lethal dose fails to produce symptoms of intoxication. In the course of this investigation certain interesting features in the action of sodium citrate were observed and interpreted in the light of further experiments which are also described in this paper.

In order to accomplish the prime object of the investigation, however, it was obviously necessary to ascertain the magnitude of a dose which may be safely regarded as being invariably toxic for a normal animal. Accordingly the following experiments were undertaken:

Doses consisting of 5, 10 or 15 cc. of M/1 sodium citrate¹ were administered subcutaneously to rabbits with the following results:

¹ The sodium citrate employed in all of the experiments was Kahlbaum's tri-sodium citrate.

. TABLE 1

DATE OF ADMINISTRATION	RABBIT	WEIGHT	DOSE OF AN M/1 SOLUTION	SYMPTOMS
	<i>number</i>	<i>grams</i>	<i>cc.</i>	
October 23.....	1	3250	10	Very severe
October 25.....	2		10	Severe
October 28.....	3	3120	5	None
October 28.....	3	3120	15	Severe
October 28.....	4	1850	15	Very severe (Death)
October 28.....	5	2500	10	None
October 29.....	6	2600	5	Slight
October 29.....	7	2000	5	Slight
November 1.....	8		15	Severe
November 1.....	1	3250	10	Very Severe
November 1.....	9		5	None
November 1.....	10		5	None
November 1.....	11		5	None (on repeating the dose on the following day moderate symp- toms were observed).

It is evident that the resistance of different animals to intoxication by subcutaneous administration of sodium citrate is very variable and bears no definite relation to their weight. A dose of 5 cc. of an M/1 solution, however, fails to produce severe symptoms; 10 cc. of an M/1 solution generally produces severe symptoms, while 15 cc. of an M/1 solution produces severe symptoms or even death.²

2. TOLERANCE TO SODIUM CITRATE ACQUIRED AS A RESULT OF REPEATED ADMINISTRATIONS

Three rabbits, weighing respectively 2350, 2900 and 2250 grams, received gradually increasing subcutaneously administered doses of sodium citrate daily. The following were the results observed:

² We may compare these doses with the minimum lethal dose of tri-sodium citrate for rabbits determined by Sabbatani (Arch. Ital. de Physiol. 44, 1905, p. 406) who administered the citrate intravenously. According to this investigator the minimal lethal dose is 0.0044 gram—equivalents per kilogram body-weight, corresponding with 13 cc. of an M/1 solution for an animal weighing 3000 grams. Administering per os Mitscherlich (cited after von Vietinghoff-Scheel, Arch. Internat. de Pharmacodynamie 10, 1902, p. 154) found about 5 grams of citric acid to be a fatal dose for rabbits, corresponding with 26 cc. of an M/1 solution of tri-sodium citrate.

TABLE 2

DATE	ANIMAL WEIGHING			SYMPTOMS
	2350 grams	2900 grams	2250 grams	
	<i>dose</i>	<i>dose</i>	<i>dose</i>	
October 11.....	5cc. M/6	5cc. M/6	5cc. M/6	None
October 12.....	5cc. M/6	5cc. M/6	5cc. M/6	None
October 13.....	5cc. M/6	5cc. M/6	5cc. M/6	None
October 14.....	5cc. M/6	5cc. M/6	5cc. M/6	None
October 15.....	10cc. M/6	10cc. M/6	10cc. M/6	None
October 16.....	10cc. M/6	10cc. M/6	10cc. M/6	None
October 17.....	10cc. M/6	10cc. M/6	10cc. M/6	None
October 18.....	10cc. M/6	10cc. M/6	10cc. M/6	None
October 19.....	20cc. M/6	20cc. M/6	20cc. M/6	None
October 20.....	20cc. M/6	20cc. M/6	20cc. M/6	None
October 21.....	20cc. M/6	20cc. M/6	20cc. M/6	None
October 22.....	20cc. M/6	20cc. M/6	20cc. M/6	None
October 23.....	10cc. M/1	10cc. M/1	10cc. M/1	None
October 24.....	10cc. M/1	10cc. M/1	10cc. M/1	None
October 25.....	(Experiment discontinued)	10cc. M/1	10cc. M/1	None
October 26.....		10cc. M/1	10cc. M/1	None
October 27.....		10cc. M/1	10cc. M/1	None
October 28.....		15cc. M/1	15cc. M/1	Slight
October 29.....		15cc. M/1	15cc. M/1	None
October 30.....		15cc. M/1	15cc. M/1	None
October 31.....		15cc. M/1	15cc. M/1	None
November 1.....		(Experiment discontinued)	15cc. M/1	None

It is evident that as a result of administering repeated and gradually increasing doses of sodium citrate these animals acquired such a pronounced degree of tolerance for the drug that a dose (15 cc. M/1) which normally causes extremely severe symptoms of intoxication, or even death, causes either very slight symptoms or else no symptoms of intoxication at all.³

An endeavor to accelerate the attainment of this tolerance by increasing the dose somewhat more rapidly yielded results which were not so striking as the above, but which nevertheless pointed

³ It is perhaps almost unnecessary to state that the food supplied to all of animals experimented upon was the same, to wit oats, alfalfa and carrots. The amount of calcium (cf. below) in the diet of the tolerant animals was therefore the same as that in the diet of the normal animals.

very clearly in the same direction. These results were also complicated by the fact that the citrate solution and consequently the animals became infected towards the end of the experiment. The animals which were employed in this experiment were not weighed. They were, however, of medium size (2500 to 3000 grams). The following were the results obtained:

TABLE 3

DATE	ANIMAL				SYMPTOMS
	No. 12	No. 9	No. 10	No. 11	
	<i>dose</i>	<i>dose</i>	<i>dose</i>	<i>dose</i>	
October 28.....	5cc. M/6				None
October 29.....	5cc. M/6				None
October 30.....	5cc. M/6				None
October 31.....	5cc. M/6				None
November 1.....	5cc. M/1	5cc. M/1	5cc. M/1	5cc. M/1	None
November 2.....	5cc. M/1	5cc. M/1	5cc. M/1	5cc. M/1	No. 11 displayed moderate symptoms, the others none.
November 3.....	5cc. M/1	5cc. M/1	5cc. M/1	5cc. M/1	None
November 4.....	5cc. M/1	5cc. M/1	5cc. M/1	5cc. M/1	None
November 5.....	5cc. M/1	5cc. M/1	5cc. M/1	5cc. M/1	None
November 6.....	5cc. M/1	5cc. M/1	5cc. M/1	5cc. M/1	None
November 7.....	5cc. M/1	5cc. M/1	5cc. M/1	5cc. M/1	None
November 8.....	10cc. M/1	10cc. M/1	10cc. M/1	10cc. M/1	No. 11 severe, No. 9 slight, others none.
November 9.....	10cc. M/1	10cc. M/1	10cc. M/1	10cc. M/1	No. 12 severe, No. 10 severe, others none.
November 10....	10cc. M/1	10cc. M/1	10cc. M/1	10cc. M/1	No. 9 and No. 10 severe, others none.
November 11....	10cc. M/1	10cc. M/1	10cc. M/1	10cc. M/1	None
November 12....	10cc. M/1	10cc. M/1	10cc. M/1	10cc. M/1	No. 10 and No. 11 slight, others none.
November 13....	10cc. M/1	10cc. M/1	10cc. M/1	10cc. M/1	No. 9 slight, others none.
November 14....	10cc. M/1	10cc. M/1	10cc. M/1	10cc. M/1	None

On November 15 no. 12 was found dead. Examination showed that this animal was suffering from a pulmonary infection and that the other animals were all suffering from extensive subcutaneous infections in the neighborhood of injection; the animals were, moreover, all in very poor condition. The experiment was therefore discontinued. It is evident, however, that these

animals had acquired a very considerable degree of tolerance to sodium citrate during the course of the experiment, although this tolerance was not nearly so marked as it was in the experiments enumerated in Table 2.

It has been shown by Loeb,⁴ Friedenthal,⁵ Sabbatani,⁶ Zoethout,⁷ Garrey⁸ and others that the toxic action of citrates and other calcium-precipitants may be referred to their power of diminishing the concentration of calcium ions in the tissues by converting the calcium into a non-ionic form. The evidence for this thesis may be briefly summarised as follows:

1. The soluble salts of acids which form non-ionised salts of lime have a characteristic action upon neuro-muscular tissue irrespective of whether calcium is actually precipitated or not. This action is inhibited by excess of soluble calcium salts. The action (production of hypersensibility in nerves, of rhythmic twitchings in muscles) may also be brought about, in a less marked degree, by solutions, such as pure NaCl solution, which decrease the relative or actual proportion of calcium in tissues through replacing it by salts of other bases. This action is also inhibited by calcium. Hence the inhibiting effect of excess of soluble calcium upon the action of calcium-precipitants is not merely due to withdrawal of the calcium-precipitant from the tissue, but to partial or complete replacement of calcium in the tissue which has been robbed of calcium (Loeb, Zoethout, Garrey, etc.).

2. Different soluble salts of acids which form non-ionised salts of calcium have a similar and equal effect upon animals when administered in equimolecular doses. The sodium salts of acids of the fatty series which precipitate calcium exert the characteristic action of other calcium-precipitants, while the sodium

⁴ J. Loeb: *Festschr. f. Prof. Fick Braunschweig*, 1899, p.101; *Univ. of Chicago Decennial Publ.*, 10, 1902, p. 3; *Amer. Journ. of Physiol.*, 1901, p. 362.

⁵ H. Friedenthal: *Verhandl. der Berl. physiol. Ges.*, Nov. 1900, *Arch. f. (Anat. und) Physiol.*, 1901 p. 145.

⁶ L. Sabbatani: *Arch. Ital. de Biol.*, 36, 1901, p. 416; 39, 1903, p. 333; 44, 1905 p. 361.

⁷ W. D. Zoethout: *Amer. Journ. of Physiol.*, 7, 1902, p. 320; 10, 1904, p. 324.

⁸ W. E. Garrey: *Amer. Journ. of Physiol.*, 13, 1905, p. 186.

salts of acids of this series which do not precipitate lime (e. g., butyric acid) do not exert this action (Friedenthal).

3. The lethal doses, for rabbits, of the sodium salts of various acids which de-ionise calcium are directly proportional to the concentrations of these salts which are required to prevent the coagulation of blood, and inversely proportional to the solubilities of their calcium salts (Sabbatani).

We may therefore regard the tolerance to sodium citrate which, as we have seen, may be established in rabbits by repeated administrations, as consisting, essentially, in *tolerance to deprivation of tissue-calcium*, and the possibility is indicated that the sensitiveness of tissues to lack of calcium is partly a phenomenon of "Unterschiedsempfindlichkeit,"—a function of the *suddenness* with which calcium is withdrawn as well as of the absolute amount of which the tissues are deprived. Just as an organism can accustom itself to several different "nitrogen levels" and maintain nitrogen equilibrium at different periods with very different nitrogen outputs, so we may imagine that the tissues affected by sodium citrate can function at various "calcium levels," provided that the transition from a higher level to a lower is sufficiently gradual. In this connection we may cite the observation of Bottazzi,⁹ Friedenthal and Sabbatani that the letheldose (intravenous injection) of a calcium-precipitant is greatly augmented if it is slowly injected, and diminished if it is rapidly injected. On the other hand it is possible that the tolerance to deprivation of tissue-calcium which is brought about by repeated administrations of sodium citrate is due to the dissolution of reserves of calcium which are ordinarily untouched but which, under the influence of repeated administrations of citrate, become available to partially replace the calcium as rapidly as it is withdrawn by a fresh dose of citrate. From this point of view we may cite the observations of Martin,¹⁰ Guenther¹¹ and Bancroft¹²

⁹ F. Bottazzi: Riv. di Scienze biologiche, 2, 1900; cited after Sabbatani, Arch. Ital de Biol., 44, 1905, p. 361.

¹⁰ Martin: Amer. Journ. of Physiol., 11, 1904, p. 103.

¹¹ A. E. Guenther: Amer. Journ. of Physiol., 14, 1905, p. 73.

¹² F. W. Bancroft: Journ. of Physiol., 39, 1909, p. 15.

who adduce evidence in favour of the view that under certain conditions, in muscular tissue, a spontaneous conversion of calcium from an unavailable to an available form may occur. The fact that prolonged administration of at least one calcium precipitant, to wit oxalic acid, leads to a pronounced diminution in the content of lime in the bones¹³ may also be of importance in this connection.

Yet another explanation which may be suggested is that tolerant animals have acquired unusual power to oxidise the citrate and render it harmless. In view of the rapidity with which intoxication normally occurs after administration, however, this explanation would appear somewhat improbable.

3. THE SYMPTOMS OF ACUTE INTOXICATION BY SODIUM CITRATE

The symptoms of acute sodium citrate intoxication in Mice, Guinea-pigs and Rabbits are described by von Vietinghoff-Scheel¹⁴ as consisting in muscular tremors and fibrillar contractions, clonic and tonic convulsions, tetany, salivation, paresis and dyspnoea, followed, in the case of lethal doses, by depression and collapse. Pommer (cited after von Vietinghoff-Scheel) observed acceleration of the respiratory movements, and Mitscherlich (cited after Vietinghoff-Scheel) observed convulsions, "vibrations" of the skeletal muscles and opisthotonus.

In human beings the symptoms of acute citrate intoxication are described by Lewin¹⁵ as consisting in headache, giddiness, neuralgia and epileptiform convulsions. Kionka (cited after Kobert, loc. cit.) describes a case in which death occurred without convulsions, but he also points out that the administration of a very large dose to rabbits (per os) may also lead to collapse and death without preliminary convulsions, while the administration of a smaller, but still lethal dose leads to marked convulsions.

In the course of the experiments described above we had many

¹³ W. Caspari: Biedermann's Centralbl. f. Agricultur-chemie, 26, p. 529. Cited after Maly's Jahresber. der Tier-chemie, 27 (1897), p. 711.

¹⁴ E. F. von Vietinghoff-Scheel: Arch. Internat. de Pharmacodynamie et de Therapie, 10, 1902, p. 145.

¹⁵ Cited after R. Kobert: Lehrbuch der Intoxikationen, 2te Aufl., Bd. I, p. 113.

opportunities of observing the symptoms of acute citrate-intoxication in rabbits. The following is a typical experiment:

A rabbit (no. 1, October 23, table 1) received 10 cc. M/1 sodium citrate solution subcutaneously at 12.35 p. m. At 12.55 p.m. nodding movements of the head and chewing movements of the jaws were observed. The animal was now placed upon the floor. It made a series of vigorous movements in which decided lack of coördination was displayed. In jumping forwards the hind legs were thrust out vigorously, but instead of the usual quick flexion they were then dragged along in an extended position. The left limbs seemed to be weaker than the right (the left was the side upon which the injection was made) and this gave rise to movements in a circle to the left. Occasionally the left side seemed to give way, and the rabbit would partly roll over to the left. The whole series of movements were disorderly. Finally the animal became quiet and lay inclined to the right. Eighty minutes after the injection the animal appeared to have completely recovered.

Characteristic symptoms which were displayed by this and the other animals experimented upon were the following:

1. The forward thrust of the fore-limbs, which were usually stretched out in front of the animal and became rigid on thrusting them or pinching the muscles of the upper part of the limb.

2. The hind legs were dragged behind in an extended position, an attitude which was not due to paralysis of these limbs but to predominance of the extensors. On pinching the muscles of the upper part of the hind limb the limb would exhibit tetanic rigidity.

3. The head was drawn back in a strained position and exhibited nodding and rolling movements.

4. Symptoms of increased secretion in various glands, such as the salivary glands, and increased secretion in the bronchial mucous membranes as evidenced by marked coarse râles.

5. Quiverings of the skeletal muscles, particularly those of the jaws.

6. Chewing movements of the jaws.

These symptoms were supplemented in the later stages of intoxication by the following:

7. Loss of sensibility and absence of reflexes.

8. Dyspnoea.

These symptoms point very clearly to excitation of the central nervous system. Moreover our colleague, Dr. S. S. Maxwell, to whom our sincere thanks are due, pointed out to us that the symptoms involve loss of co-ordination and equilibrium to so marked a degree as to strongly suggest that many of the symptoms of acute citrate-intoxication may be directly attributable to excitation of the cerebellum. Accordingly the following experiments were undertaken with a view to ascertaining to what extent the characteristic symptoms enumerated above could be elicited by local application to the cerebellum.

4. THE ACTION OF SODIUM CITRATE UPON THE CEREBELLUM

Sabbatani (*loc. cit.*), Roncorini,¹⁶ Regoli¹⁷ Maxwell¹⁸ and Robertson¹⁹ have applied calcium-precipitants directly to various parts of the central nervous system other than the cerebellum. Sabbatani obtained intense excitation and violent tetany on applying sodium citrate to the spinal cord.²⁰ Roncorini and Regoli observed an increase in the excitability of the cerebral cortex (or, as Maxwell's experiments show, of the underlying white matter) on applying calcium precipitants. Maxwell found that application of citrate to the cerebral cortex in rabbits, or its injection into the cortex itself was without effect, but immediately, whether by diffusion or by direct injection, the calcium-precipitant reached the underlying white matter, convulsive movements were observed. Robertson obtained analogous results in applying sodium oxalate or oxalic acid to the medulla in frogs.

As regards the effects of stimulating the cerebellum the following results, obtained by previous observers, may be cited. Pagano²¹

¹⁶ L. Roncorini: *Arch. d. Psichiatria*, 24, 1903; cited after Sabbatani, *loc. cit.*

¹⁷ P. Regoli: *Boll. della Soc. tra i cultori di Sc. Med. Nat. in Cagliari*, 1899-1900, p. 151; cited after Sabbatani, *loc. cit.*

¹⁸ S. S. Maxwell: *Journ. Biol. Chem.*, 2, 1906, p. 183.

¹⁹ T. Brailsford Robertson: *Arch. Internat. de Physiol.*, 6, 1908, p. 388.

²⁰ A result also obtained by Garrey (*loc. cit.*) working upon Frogs.

²¹ G. Pagano: *Arch. Internat. de Physiol.*, 2, 1894, p. 134; *Revista di Patologia nervosa e mentale*, 9, p. 209; cited after van Rynberk (*cf. below*).

injected 0.1 cc. of a 1 per cent solution of curare into various regions of the cerebellum of dogs. Injection into the anterior portion of the worm caused the dog to spring forward, barking and howling. The dog leapt into the air, ran against the wall, attempted to bite, appeared the victim of all sorts of hallucinations and exhibited a veritable "motor delirium."

Nothnagel in 1876²² made a number of "mechanical excitation" experiments upon the cerebellum of the rabbit. He injured various portions of the cerebellum with a needle and obtained rhythmic up and down movements of the forefeet, clapping together of the jaws and opisthotonus.

Beck and Bikeles²³ applied pieces of paper soaked in 1 per cent and 2 per cent strychnin solution upon various parts of the cerebellum, with negative results. They also obtained negative results in applying 1 per cent or 3 per cent phenol solutions in a similar manner. They conclude that "die Kleinhirnrinde als solche nicht erregbar ist."

Our own experiments follow:

FIRST EXPERIMENT

The surface of the cerebellum in a rabbit was exposed and the adhering dura mater carefully removed. After allowing about three hours to elapse, in order to enable the animal to recover from the effects of the anaesthetic, a cotton plug drenched in M/6 sodium citrate solution was applied directly to the surface of the cerebellum. No effect was observed after ten minutes. A fresh cotton plug was then applied which had been drenched in M/1 sodium citrate; no effects were observed after fifteen minutes.

We now injected 1 to 2 drops of M/6 sodium citrate solution a few millimeters below the surface of the cerebellum on the left side. Instantly the animal dashed forward and performed a series of violent running and leaping movements accompanied by rotation to the left. The animal then leapt 3 feet into the

²² Cited after van Rynberk: *Ergeb. d. Physiol.*, 7, 1908, p. 681.

²³ Beck and Bikeles: *Zentr. f. Physiol.*, 25, 1911, p. 1066.

air and fell flat upon its left side. After a brief period of rigid extension very characteristic pawing movements of the fore-legs were observed. Within thirty seconds the animal had apparently recovered completely, exhibiting no symptoms whatever beyond a slight restlessness.

After a lapse of some five minutes we gave the animal a second injection, this time of from 0.2 to 0.5 cc. on the *right* side of the cerebellum. The animal gave a short rush forward, *curving to the right*, and instantly fell upon its right side with violent leaping movements. Transient tetany and opisthotonus then occurred, followed by violent pawing movements of the fore- and hind-legs which persisted until death, the left fore-leg being crossed over the right. The animal suffered from marked dyspnoea. When laid upon its left side it turned over and fell upon its right side. In seven minutes respirations and the heart-beat ceased. Within six minutes after death pronounced rigor mortis had set in.

From this experiment it is evident that, as in Maxwells' experiments upon the cerebral cortex and the experiments of Beck and Bikeles cited above, the cortex of the cerebellum is not affected by nerve-stimulants, while the application of the stimulant to the underlying white matter leads to a profound reaction.

SECOND EXPERIMENT

The following experiment confirms the above and also shows that the effects observed were not due to the mechanical lesion produced by the injection of the citrate into the cerebellar white matter.

The surface of the cerebellum in a rabbit was exposed and the adhering dura mater carefully removed. After allowing several hours to elapse about 2 drops of Ringer-solution were injected a few millimeters below the surface of the cerebellum near the middle and a little towards the left. No effect whatever was observed after five minutes.

We now injected 4 drops of M/6 sodium citrate a few millimeters below the surface of the cerebellum on the left side.

The animal instantly dashed forward, *curved to the left*, and fell on the right side. Convulsive movements, tetany and slight opisthotonus, lasting about fifteen to thirty seconds, were followed by typical pawing movements of the legs as in the previous experiment. The respirations became very rapid, in three minutes after the injection the rate being 168 per minute. The hind-legs, when touched or pushed, became rigid and ceased "pawing." The breathing and the pawing movements were synchronous. There was no forced position of the eyes. When placed upon its left side the animal did not turn over. In six minutes moist râles were heard in the throat and the pawing movements were becoming weak.

In twelve minutes the respirations were 116 per minute; the râles had ceased and the pawing movements had stopped; the animal was still prone and exhibited marked loss of sensibility. On thrusting a fore-foot very slightly, it became rigid for from ten to fifteen seconds and then was sharply pulled away and braced up against the side.

In seventeen minutes the reflexes began to re-appear. The animal was now able to rest upon the floor with the fore-paws both upon the ground and the hind-legs extended. We now were able to observe a very marked tendency for the head to slew round, rotating around a horizontal antero-posterior axis to the left (the side of injection) and around a vertical axis to the right. This was repeated again and again, even when the animal was placed in a semi-prone position upon its right side. In thirty minutes the animal had recovered to all appearances, and it was left until the following day.

Upon the following day we injected 4 drops of Ringer-solution a few millimeters below the surface of the cerebellum upon the right side. No effect was observed after four minutes. We then injected from 2 to 4 drops of M/6 sodium citrate solution into the same region. The animal instantly gave a forward rush, made two complete rotations *to the right* and then fell upon its right side in a condition of tetany with opisthotonus; during this period the respiratory movements stopped. In thirty seconds the typical pawing movements accompanied by respirations

appeared, but they were much weaker than they were upon the previous day. A slight tendency was observed for the head to rotate to the *right* about a horizontal antero-posterior axis. Reflexes could not be elicited.

In three minutes a second dose of 4 drops of M/6 sodium citrate solution was injected a few millimeters below the surface of the cerebellum in the centre. No additional effect was observed beyond gasping accompanied by sharp opening and closure of the jaws and slight salivation. The breathing movements became very slow and labored and in ten minutes ceased suddenly. Rigor mortis set in in the hind legs almost at once and in the fore-legs within a few minutes.

From these experiments it is evident that with the exceptions of the chewing movements (probably attributable to cerebral excitation, cf. Maxwell loc. cit.) and of the quiverings and twitchings of the skeletal muscles, which are probably peripheral in origin, all of the symptoms which are described in part 3 as being characteristic of acute citrate-intoxication in rabbits, produced by the subcutaneous administration of large doses, can be elicited by the direct application of minute doses of sodium citrate to the white matter of the cerebellum. It is probable therefore that all of the symptoms of acute citrate-intoxication following the subcutaneous administration of large doses, with the exceptions noted, *are attributable to direct cerebellar excitation by the citrate.* We may also conclude that of the various parts of the central nervous system the cerebellum is the one which is most sensitive to the excitant action of deprivation of calcium.

CONCLUSIONS

1. As a result of administering repeated and gradually increasing doses of sodium citrate, rabbits acquire such a pronounced degree of tolerance for the drug that a dose (15 cc. M/1) which normally causes extremely severe symptoms of intoxication, or even death, causes either very slight symptoms or else no symptoms of intoxication at all.

2. This tolerance is to be regarded as consisting, essentially, in tolerance to deprivation of tissue-calcium.

3. Two alternative explanations of this phenomenon are suggested, namely:

(a) The sensitiveness of tissues to lack of calcium is partly a phenomenon of "Unterschiedsempfindlichkeit." Just as an organism can accustom itself to several different "nitrogen levels" and maintain nitrogen equilibrium at different periods with very different nitrogen outputs, so we may imagine that the tissues affected by sodium citrate can function at various "calcium levels" provided the transition from a higher level to a lower is sufficiently gradual.

(b) Tolerant animals are able to draw upon reserves of calcium which are ordinarily not available.

4 The cortex of the cerebellum, in rabbits, is not affected by the direct application of sodium citrate to its surface, while the application of sodium citrate to the underlying white matter elicits a profound reaction.

5. With exceptions noted in the text the symptoms of acute citrate-intoxication in rabbits, following the subcutaneous administration of large doses, are attributable to direct cerebellar excitation by the citrate, inasmuch as they may be elicited by the direct application of minute doses to the white matter of the cerebellum.

6. Of the various parts of the central nervous system the cerebellum is the one which is most sensitive to the excitant action of deprivation of calcium.

THE ACTION OF SALTS OF CHOLINE ON ARTERIAL BLOOD PRESSURE

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The discovery of the presence of choline in many of the animal tissues either preformed or as a derivative of larger complexes, and the possible relationship of this base to some of the so-called "hormone" effects in the organism has lent a new interest to the precise determination of its physiological rôle. The contention that absolutely pure choline salts fail to induce the characteristic fall in blood pressure commonly described as a typical effect of the injection of this compound was supported several years ago by Popielski and his pupil Modrakowski.¹ They believed that pure choline causes only a rise of pressure; and they maintain that those investigators who report a fall of pressure have worked with impure or deteriorated preparations. This contradiction of the customary teaching regarding the physiological action of choline naturally elicited a speedy reinvestigation of the question, to which we, among others, contributed a preliminary reply in 1910.² One might assume that our experience, together with the experimental studies of Abderhalden and Müller,³ Lohmann,⁴ Berlin,⁵

¹ Modrakowski: Pflüger's Archiv für die gesammte Physiologie, 1908, exxiv, p. 601.

² Mendel and Underhill: Zentralblatt für Physiologie, 1910, xxiv, p. 251 (June 25).

³ Abderhalden and Müller: Zeitschrift für physiologische Chemie, 1910, lxxv, p. 420; 1911, lxxiv, p. 253.

⁴ Lohmann: Zeitschrift für Biologie, 1911, lvi, p. 1.

⁵ Berlin: Zeitschrift für Biologie, 1911, lvii, p. 1.

Hunt and Taveau⁶—to mention only a part of those which have arisen—had adequately controverted the newer claims and restored confidence in the earlier view point. However, the continued reiteration of Popielski's allegations in recent papers⁷ induces us to present a few further details of our trials.⁸ They were carried out entirely with synthetic preparations made by Dr. Renshaw at Middletown according to the improved method devised by him.⁹ Since the controversy centers largely in the purity of the salts used, our attention has been devoted primarily to this point. We have been unable to confirm Popielski and Modrakowski's claims respecting the absence of depressor effects when the purity of the choline products is sufficiently assured.

PREPARATIONS OF CHOLINE USED—METHODS

Eight different preparations of choline chloride and sulfate were tested in numerous trials. The animals (usually cats) were maintained in deep ether anaesthesia. The importance of this deserves the emphasis which Abderhalden and Müller have given to it; for when the narcosis is not adequate it is easy to obtain transitory rise of pressure reflexly by even slight manipulation of the animal prior to the act of intravenous injection. Unless specifically stated otherwise, the choline salts were dissolved in 0.9 per cent NaCl solution just prior to the injection into an exposed vein. Popielski remarks: "Die Schwierigkeit der physiologischen Untersuchung des Cholins ist auf die grosse Schwierigkeit der Gewinnung von chemisch reinen Praeparaten zurückzuführen

⁶ Hunt and Taveau: Bull. No. 73. Hygienic Laboratory, U. S. Pub. Health and Mar. Hosp. Service, Washington, 1911, p. 12. Details of the literature will be found in this paper and in the contributions of Abderhalden and Müller, so that they need not be repeated here. Cf. also Gautrelet: Journal de physiologie, 1909, xi, p. 227; v. Fürth: Probleme der physiologischen und pathologischen Chemie, 1912, p. 186, ff.

⁷ Cf. Studinski: Archiv für experimentelle Pathologie und Pharmakologie, 1911, lxxv, p. 155 (Popielski's laboratory); also Samelson: *ibid.*, 1912, lxxvi, p. 347.

⁸ A report was presented to the American Society for Pharmacology and Experimental Therapeutics at the meeting in December, 1911. Cf. Journal of Experimental Pharmacology and Therapeutics, 1912, iii, p. 457.

⁹ Renshaw: Journal of the American Chemical Society, 1910, xxxii, p. 128; Abderhalden's Biochemisches Handlexikon, 1911, iv, p. 829.

ren.”¹⁰ Accordingly we shall describe some of our products in sufficient detail to permit the reader to form a more critical judgment as to their probable purity.

Choline chloride: Preparations 1, 2 and 3

Preparation 1 was the product of the *sixth* reprecipitation of an alcoholic solution of our synthesized material with ether. Not more than one-third of the dissolved product originally used was removed from the solution, thus creating ideal conditions for the exclusion of soluble impurities. An analysis gave:

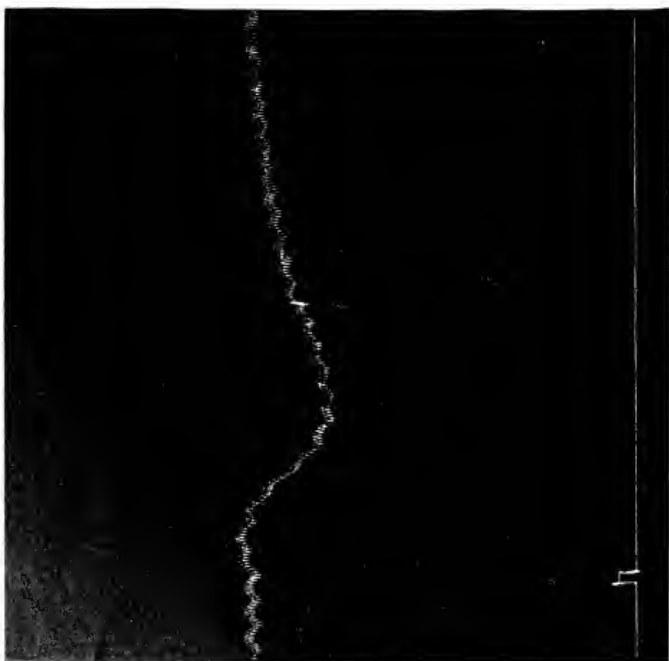
Cl. found 25.45 per cent, 25.49 per cent; calculated, 25.41 per cent

Preparation 2 was obtained after five additional reprecipitations of 1.

Preparation 3, our purest specimen, was the product of the *fifteenth* reprecipitation.

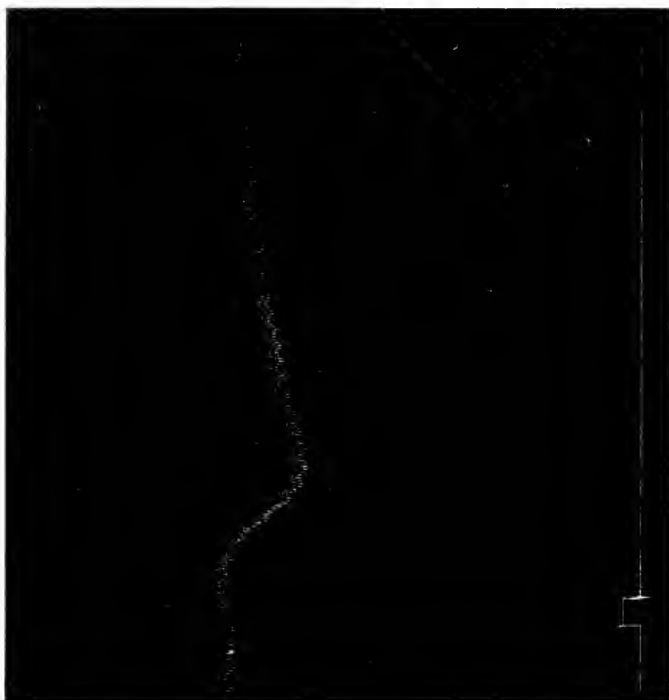
The second of three partial precipitations on both the first and the second solutions of the choline chloride was taken as the sample for purification. From the third on the procedure was as indicated. The reason was, of course, to eliminate the possibility of any amount of a somewhat more insoluble product always precipitating with the chloride. Had such a substance been present even in fair amount this procedure would have eliminated all but such quantities as the solvent could have taken care of. Such a possibility was very remote; but on account of the controversy it seemed worth while to eliminate every point that might be criticised. None of these preparations had any odor of trimethylamine, nor did they develop it rapidly when exposed to sunlight. This is important in relation to the alleged speedy deterioration of choline salts discussed later. In view of the differences in the extent of purification attempted one would expect wide variations in the degree of purity and a consequent difference in physiological action dependent upon the amount of contaminating impurity; i.e., preparation 3 ought (according to the

¹⁰ Popielski: Zeitschrift für physiologische Chemie, 1910, lxx, p. 250.



CAT. 2 KGM. INJECTION OF 0.6 MGM. (0.3 CC.)

Preparation 1



Preparation 3

explanation of depressor effects on the impurity hypothesis) to be less active physiologically than 1 or 2.

These preparations were delivered in sealed tubes and injected the same day. *Twenty-one months later* the experiments with these products were duplicated, the preparations having been kept meanwhile in glass-stoppered bottles in a dark desiccator. There is no evidence for any lack of, or quantitative differences in, the depressor effects of measured doses of choline chloride 1, 2 or 3; nor has the long time interval noticeably altered the behavior of the purest product, 3, despite Modrakowski's contentions of the extreme instability of choline salts.

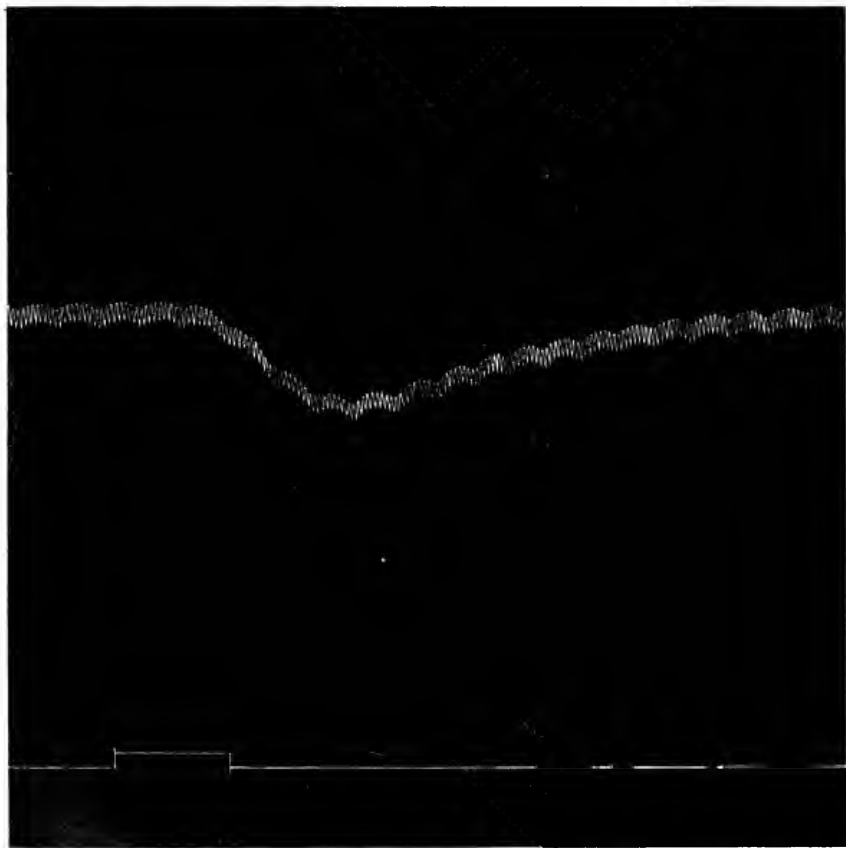
The illustrative blood pressure tracings should be read from left to right. A few of the numerous data are summarized in tabular form in the Appendix. It is unnecessary to report the observations with larger doses, since they were not essentially different in character. Furthermore the fact that a fall was always produced even with the very small doses is significant of itself.

Comparative effects of choline chloride 1 (recrystallized six times) and 3 (recrystallized fifteen times) on blood pressure. The preparations were not more than twenty-four hours old

Further evidence of the failure of this pure product to develop any extreme toxicity on standing was shown by experiments made with choline chloride 3. The solution was prepared one month after the preparation of the salt and then *allowed to stand thirty-two days in the laboratory before being tested*. Surely here was abundant opportunity for the development of the extreme depressor effects assumed to be characteristic of old preparations and depicted in the curves of other investigators for "crude" choline. The tracing shows merely the typical transitory fall regularly found when the same product was used *immediately* after its delivery.

Choline chloride 3—old solution

This curve may also be compared with that obtained in 1910 with the fresh preparation. (Cf. *Zentralblatt für Physiologie*, 1910, xxiv, p. 252.)



CAT. 2.8 KGM. INJECTION OF 2.4 MGM. (0.8 CC.)

The solution stood one month in the laboratory before being used.

Similar depressor results were obtained on dogs with the fresh purest choline chloride 3, even with doses of 0.1 mgm. per kilogram. The salt was used within thirty hours after it was made.

Choline sulfate

A pure preparation of this salt¹¹ was likewise examined. The depressor action was never missed even in small doses. It was not found increased when the same preparation, carefully preserved, was tested three months later; i.e., there was no evidence of a production of the hypothetical depressor derivative on standing.

Since Modrakowski has especially emphasized the method of Gulewitsch¹² as essential to obtain *pure* choline we have also followed this plan of purification.

A solution of choline chloride was evaporated until no amine odor was detectable. It was converted into the platinic salt, recrystallized five times, decomposed with hydrogen sulfide, and finally obtained as a pure crystalline chloride = *choline chloride 4*.

This salt, injected within a day after its preparation gave the usual characteristic fall of pressure, as reported likewise by Abderhalden and Müller and by Lohmann for products similarly purified.

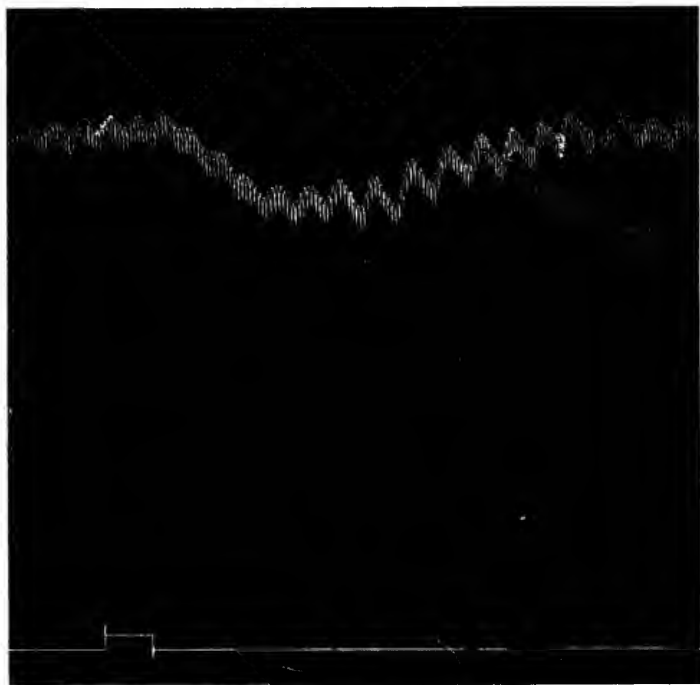
Inasmuch as the hypothetical "impurities" which are alleged to account for the fall in pressure are assumed to be eliminated by this process of purification we searched for them in the wash solutions or mother liquors of choline chloride 4. They were precipitated with ether. The new product, *choline chloride 5* in which the decomposition product might be concentrated, might be expected to show the depressor effects in marked degree. This was, however, not the case. The result was merely the typical transitory fall of arterial pressure characteristic of similar doses of the purest choline salts.

It occurred to us that possibly the manipulations to which Modrakowski subjected his solutions—the evaporations to remove the amine odor, the treatment with hydrogen sulfide, a possible failure to remove the last traces of platinic sulfide which separate with difficulty—might have developed some antagonistic substance and thus "masked" the typical depressor effect despite the

¹¹ Cf. Renshaw: *Journal of the American Chemical Society*, 1910, xxxii, p. 129.

¹² Gulewitsch: *Zeitschrift für physiologische Chemie*, 1898, xxiv, p. 513; Cf. Modrakowski: *Pflüger's Archiv für die gesammte Physiologie*, 1908, cxxiv, p. 619.

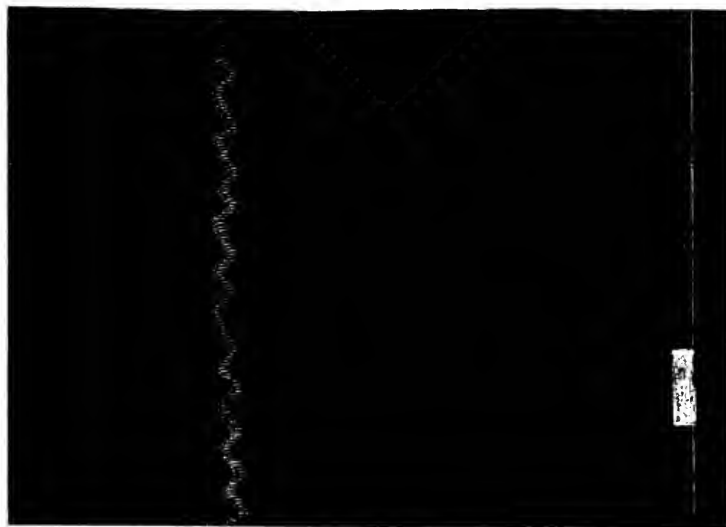
chemical purification of the choline salts. Solutions of our preparations were accordingly evaporated and treated with hydrogen sulfide alone without altering the physiological effects obtained. One solution was evaporated to remove the amine odor completely, recrystallized, redissolved in alcohol, and reprecipitated with ether five times. A blood pressure tracing of this *choline chloride 6* made within twenty-four hours is reproduced here.



CAT. 2.8 KGM. INJECTION OF 0.5 MGM. (0.5 CC.)

Choline chloride 6—repeatedly purified

That traces of platinic salts do not alter the results and thus account for the alleged rise of pressure was plainly shown by numerous trials with varying proportions of the heavy metal added.



CAT. 2.7 KGM. THE ANIMAL HAD RECEIVED 1 CC. $\frac{1}{4}$ PER CENT SOLUTION OF ATROPINE SULFATE SEVEN MINUTES EARLIER
Injection of 1.5 mgm. (0.5 cc.) choline sulfate.



CAT. 2.7 KGM. THE ANIMAL HAD RECEIVED 1 CC. $\frac{1}{4}$ PER CENT SOLUTION OF ATROPINE SULFATE SEVEN MINUTES EARLIER
Injection of 6.2 mgm. (2 cc.) choline sulfate.

OTHER FACTORS

Modrakowski has maintained (p. 620) that the characteristic blood pressure effect of pure choline is precisely comparable with that obtained from "impure" preparations after atropine is administered. We have found that atropine always abolishes the depressor effects of choline salts; but according to our experience a rise in pressure under these conditions is only obtained when the doses of choline salts administered are relatively large. This is shown in the appended tracings.

Choline sulfate following atropine

Section of the vagi does not alter the effect of pure choline salts.

It is only fair to point out that our preparations do not exhibit any of the extreme depressor effects, with attendant symptoms, which have sometimes been described by those who have used crude commercial products. Modrakowski has pointed out, for example, that the latter in doses of 1 to 3 mgm. per kilogram lead to prolonged inhibition of the heart beat and that repeated injections may impair the peripheral endings of the vagi and be followed by rise in pressure. "Bei rasch hintereinander erfolgenden Injektionen vermag die blutdrucksteigernde Cholinwirkung doch durchzudringen" (p. 620). We have never obtained more than a transitory fall of pressure even with larger doses of our choline preparations than are reported in this paper. On the other hand a rise of pressure was never observed (except after the use of atropine) even when many repeated injections were undertaken.

The deterioration of choline salts on standing

Our experience throws some light upon this question which is obviously of moment in the controversy at hand. In pointing out the extraordinary instability of even pure salts of choline Modrakowski wrote (p. 622): "Das Kahlbaum'sche Präparat war, wie bereits erwähnt, bei seinem Eintreffen vollkommen frei von Trimethylamingeruch. Gelegentlich einer kurzen Unterhaltung im Laboratorium hielt ich dasselbe einige Augenblicke in der Sonne; da erregte der Umstehenden und meine Aufmerksamkeit ein deutlicher Trimethylamingeruch, welcher der halboffenen Flasche,

die die Cholinchloridkrystalle enthielt, entströmte. Es zeigte sich also, dass unter geeigneten Bedingungen die Zersetzung dieses Präparates fast momentan erfolgen kann. Die Möglichkeit erschien daher äusserst wahrscheinlich dass kürzere oder längere Aufbewahrung die Wirkung des Cholins verändern könnte." Lohmann, on the other hand, insists that choline chloride does not decompose even when exposed to the direct sunlight for months.¹³ This is in accord with experience of Dr. Renshaw in similar trials. Abderhalden and Müller are uncertain as to whether Kahlbaum's preparations are contaminated or undergo secondary decomposition. Their own synthetic chloride gave no odor of trimethylamine after being preserved sealed in the dark for six months. "Wir möchten uns aus diesen Gründen vorläufig nicht darüber äussern, ob ganz reines Cholinchlorhydrat, unter geeigneten Bedingungen (verschlossen, unbelichtet), aufbewahrt, *unbegrenzt* haltbar ist."¹⁴ We have failed to note a development of odor after two years in preparations thus preserved. In any event no deterioration is detectable, as already pointed out, from the standpoint of quantitative alterations in blood pressure effects which, after all, Modrakowski used as his chief criterion.

CONCLUSIONS

The experiments recorded above, along with numerous further records, afford added evidence that the views promulgated by Popielski and his pupils about the physiological behavior of salts of choline are not tenable. Even with exceptionally pure synthetic salts we have never failed to observe the characteristic transitory fall of arterial pressure—a fall not profound or prolonged, but never absent even when fractions of a milligram of purest products are injected. The "contamination" theory is rendered improbable by the fact that choline salts showed no quantitative differences in the physiological effect when different specimens of presumably unequal purity were investigated. Furthermore, it seems extremely doubtful if properly prepared and preserved choline salts readily decompose.

¹³ Lohmann: *Zeitschrift für Biologie*, 1911, lvi, p. 16.

¹⁴ Abderhalden and Müller: *Zeitschrift für physiologische Chemie*, 1911, lxxiv, p. 264.

APPENDIX

Selected protocols of blood pressure experiments with salts of choline (only trials with small dosage are reported here)

PREPARATION USED	DOSE PER KILOGRAM	FALL OF PRESSURE AFTER THE INJECTION	REMARKS
	<i>mgm.</i>	<i>mm. Hg.</i>	
Choline chloride 1.	0.1	4	This salt was recrystallized six times. See tracing I
	0.3	26	
	0.5	36	
	0.1	5	This salt was recrystallized fifteen times. See tracing I
	0.3	22	
	0.5	30	
Choline chloride 3.	1.0	24	See tracing in Zentbl. Physiol., 1910, xxiv, 252.
	0.23	26	After being preserved twenty-one months without deterioration
	0.45	36	The solution stood one month in the laboratory before being used. See tracing II
	0.9	26	
	0.1	6	
	0.2	28	Dog
Choline chloride 4.	0.05	15	Purified by method of Gulewitsch
Choline chloride 5.	0.35	16	Fraction which ought to contain the hypothetical depressor product in marked concentration
Choline chloride 6.	0.18	24	Repeatedly purified. See tracing III
	3.0	30	
Choline sulfate.	0.05	11	
	0.1	17	
	0.5	32	

INDEX TO VOLUME III

Abel, John J. On the action of drugs and the function of the anterior lymph hearts in cardiectomized frogs.....	581
Abel, John J., and Macht, David I. Two crystalline pharmacological agents obtained from the tropical toad, <i>Bufo</i> <i>agua</i>	319
Action of diuretics in uranium nephritis (Proceedings).....	459
Action of drugs and the function of the anterior lymph hearts in cardiectomized frogs.....	581
Action of glandular extracts upon the amount of epinephrin in the blood.....	625
Action of hydrocyanic acid on the respiration and the antagonistic action of sodium iodosobenzoate.....	131
Action of salts of choline on arterial blood pressure.....	649
Action of sodium citrate upon mammalia, with especial reference to acquired tolerance and to its action upon the cerebellum.....	635
Adrenal secretion, Note on the effect of nicotine injection on.....	379
Alcohol and caffeine: a study of antagonism and synergism.....	267
Allergic reaction of inflammatory character, The influence of sodium iodoxybenzoate and sodium cyanide upon an.....	223
Alsberg, C. L. The toxic action of <i>amianthium muscaetoxicum</i> (Proceedings).....	473
Amberg, Samuel, and Knox, J. H. Mason, Jr. The influence of sodium iodoxybenzoate and sodium cyanide upon an allergic reaction of inflammatory character.....	223
American Society for Pharmacology and Experimental Therapeutics, Scientific proceedings of the.....	455
Anaphylaxis, Physiological studies in. IV. Reaction of the cat toward horse serum.....	299
Anesthesia in plants possessing a motor mechanism and in animals, Comparison of (Proceedings).....	467
Anterior lymph hearts in cardiectomized frogs, On the action of drugs and the function of the.....	581
Antiseptic and bactericidal action of the sodium salts of iodbenzoic, iodosobenzoic and iodoxybenzoic acids, On the.....	145
Apomorphine, The seat of the emetic action of.....	551
Arkin, Aaron. On the antiseptic and bactericidal action of the sodium salts of iodbenzoic, iodosobenzoic and iodoxybenzoic acids.....	145
Atropine, The effects of pilocarpine and, upon the amylolytic power and composition of the saliva.....	1
Aub, J. C., Binger, C. A. L., and Cannon, W. B. A note on the effect of nicotine injection on adrenal secretion.....	379
Auer, J. The effect of the digitalis group including anaphylaxis upon some skeletal muscles in rabbits, especially in regard to rigor (Proceedings)....	464

Bactericidal action of the sodium salts of iodbenzoic, iodosobenzoic and iodoxybenzoic acids, On the antiseptic and.....	145
Binger, C. A. L., Cannon, W. B., and Aub, J. C. A note on the effect of nicotine injection on adrenal secretion.....	379
Biologic tests for epinephrin, A consideration of some.....	93
Blood pressure, The action of salts of choline on arterial.....	649
Bufo aqua, Two crystalline pharmacological agents obtained from the tropical toad.....	319
Burnett, Theo. C. and Robertson, T. Brailsford. On the action of sodium citrate upon mammalia, with especial reference to acquired tolerance and to its action upon the cerebellum.....	635
Caffein: Alcohol and, a study of antagonism and synergism.....	267
Caffein, The action of, on the mammalian heart.....	609
Caffein, The actions of, on the mammalian circulation.....	19
Caffein, The elimination of (Proceedings).....	469
Caffein, The persistent effects of, on the circulation.....	19
Caffein, The toxicity of (Proceedings).....	455
Cannon, W. B., Aub, J. C., and Binger, C. A. L. A note on the effect of nicotine injection on adrenal secretion.....	379
Cardiac circulation, On the convulsant action of acid fuchsin upon frogs deprived of their.....	183
Cardiectomized frogs, On the action of drugs and the function of the anterior lymph hearts in.....	581
Chloral poisoning, Chronic (Proceedings).....	462
Choline, The action of salts of, on arterial blood pressure.....	649
Choline, The physiological action of salts of (Proceedings).....	457
Circulation, The actions of caffein on the mammalian.....	19
Circulation, The persistent effects of caffein on the.....	19
Convulsant action of acid fuchsin upon frogs deprived of their cardiac circulation.....	183
Convulsant action of some sulphonated dyes.....	531
Crile, G. W., and Menten, M. L. Comparison of anesthesia in plants possessing a motor mechanism and in animals (Proceedings).....	467
Crystalline pharmacological agents obtained from the tropical toad, Bufo aqua.....	319
Cytisine, the active alkaloid of laburnum (cytissus-laburnum), The physiological action of.....	205
Dale, H. H., and Laidlaw, P. P. The physiological action of cytisine, the active alkaloid of laburnum (cytissus-laburnum).....	205
Dandy, W. E., and Rowntree, L. G. Peritoneal and pleural absorption (Proceedings).....	460
Demethylation of caffein and theobromin under pathological conditions (Proceedings).....	469
Diuretics in uranium nephritis, Study of the action of various,.....	423
Dresbach, M. The physiological action of phenolphthalein oxine.....	161
Dyes, On the convulsant action of some (Proceedings).....	471

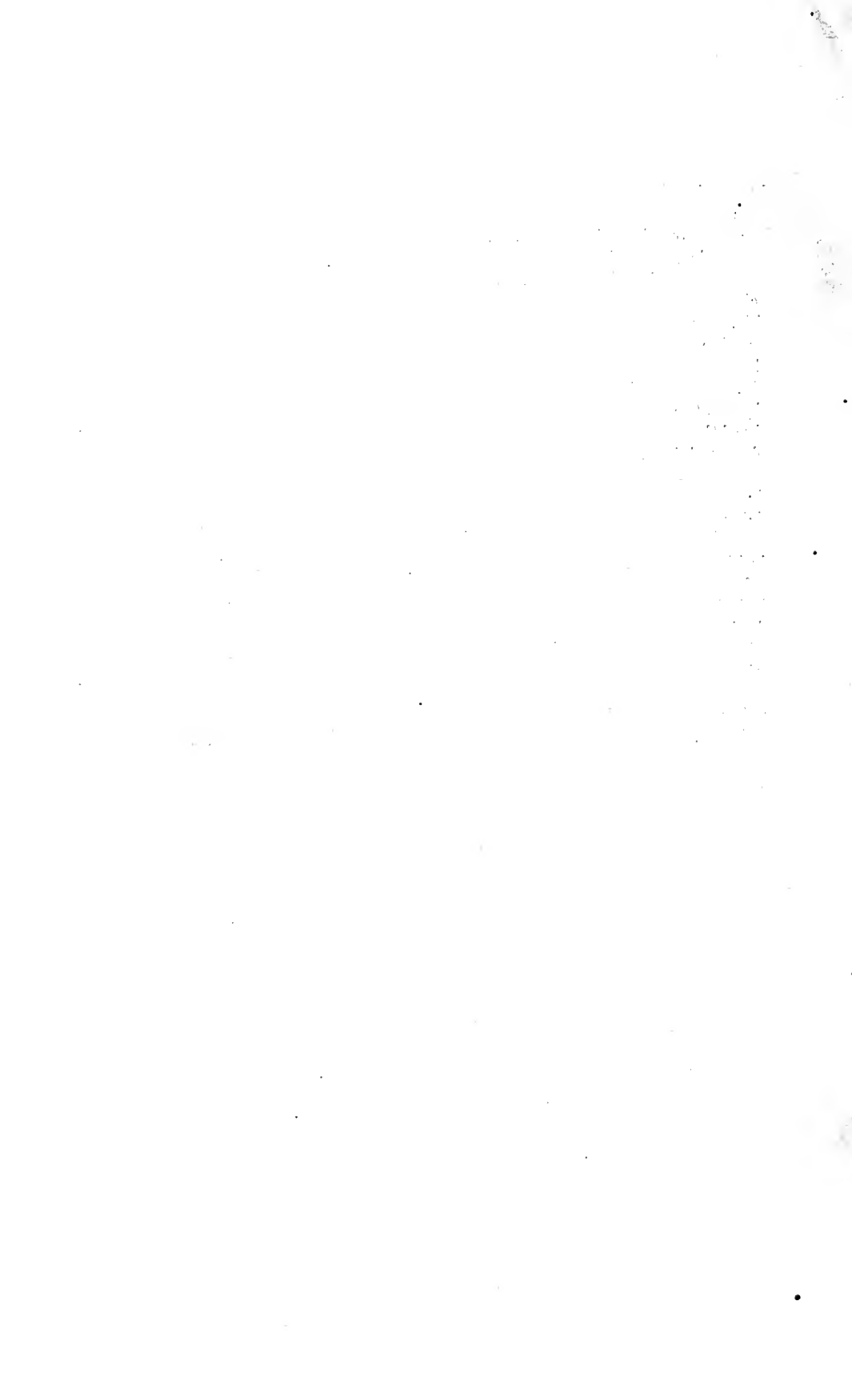
Edmunds, C. W. Upon the action of protein poison (Proceedings).....	461
Effect of caffein on the circulation (Proceedings).....	468
Effect of nicotine injection on adrenal secretion, A note on the.....	379
Eggleston, Cary, and Hatcher, Robert A. The seat of the emetic action of apomorphine.....	551
Electrocardiogram in morphin poisoning in the dog (Proceedings).....	468
Emetic action of apomorphine, The seat of the.....	551
Epinephrin, A consideration of some biologic tests for.....	93
Epinephrin in the blood, The action of glandular extracts upon the amount of.....	625
Epinephrine.....	521
Ewing, E. M. The effects of pilocarpine and atropine upon the amylolytic power and composition of the saliva.....	1
Ewins, Arthur James. A new water soluble active constituent of squills.....	155
Eyster, J. A. E., and Meek, W. J. The electrocardiogram in morphin poisoning in the dog (Proceedings).....	468
Fitz, R., and Rowntree, L. G. Studies of renal function in experimental nephritides (Proceedings).....	460
Fuchsin, On the convulsant action of acid, upon frogs deprived of their cardiac circulation.....	183
Gastro-intestinal absorption of drugs, Quantitative studies on the.....	387
Geraghty, J. T., and Rowntree, L. G. Intramuscular versus subcutaneous absorption (Proceedings).....	460
Githens, T. S., and Meltzer, S. J. On the duration of irritability of the motor nerves of the frog after death and the effect of strychnin upon it (Proceedings).....	463
Glandular extracts, The action of, upon the amount of epinephrin in the blood.....	625
Grove, W. E., and Loevenhart, A. S. The action of hydrocyanic acid on the respiration and the antagonistic action of sodium iodosobenzoate.....	131
Grove, W. E., and Loevenhart, A. S. Studies on the pharmacological action of oxidizing substances.....	101
Habit.....	515
Hale, Worth. The seasonal and sex variations of <i>Rana pipiens</i> to digitalis (Proceedings).....	458
Hanzlik, Paul J. Quantitative studies on the gastro-intestinal absorption of drugs. II. The absorption of sodium iodide.....	387
Hanzlik, Paul J. and Sollmann, T. Inhibition of iodide absorption by chloride (Proceedings).....	473
Hatcher, Robert A., and Eggleston, Cary. The seat of the emetic action of apomorphine.....	551
Hess, C. L. von, and McGuigan, Hugh. Isocalycanthine and its quaternary base.....	441
Horse serum, Reaction of the cat toward.....	299
Hoskins, R. G. A consideration of some biologic tests for epinephrin.....	93
Hydrocyanic acid, The action of, on the respiration and the antagonistic action of sodium iodosobenzoate.....	131

Intramuscular versus subcutaneous absorption (Proceedings).....	460
Intravenous and intra-aortic injections of curarin in frogs, On a difference between the effects of (Proceedings).....	465
Iodobenzoic acid, On the antiseptic and bactericidal action of the sodium salts of	145
Iodide, The absorption of sodium.....	387
Iodosobenzoic acid, On the antiseptic and bactericidal action of the sodium salts of	145
Iodoxybenzoic acid, On the antiseptic and bactericidal action of the sodium salts of	145
Irritability of the motor nerves of the frog after death and the effect of strychnin upon it, On the duration of (Proceedings).....	463
Isocalycanthine and its quaternary base.....	441
Jackson, D. E. The pharmacological action of vanadium.....	477
Joseph, D. R., and Meltzer, S. J. On the convulsant action of acid fuchsin upon frogs deprived of their cardiac circulation.....	183
Joseph, D. R., and Meltzer, S. J. On a difference between the effects of intra- venous and intra-aortic injections of curarin in frogs (Proceedings).....	465
Knox, J. H. Mason, Jr., and Amberg, Samuel. The influence of sodium iodoxy- benzoate and sodium cyanide upon an allergic reaction of inflammatory character.....	223
Laburnum (cytissus-laburnum), Physiological action of cytisine the active alkaloid of	205
Laidlaw, P. P., and Dale, H. H. The physiological action of cytisine, the active alkaloid of laburnum (cytissus-laburnum).....	205
Leucocytes, The action of quinine on (Proceedings).....	470
Loevenhart, A. S., and Grove, W. E. The action of hydrocyanic acid on the respiration and the antagonistic action of sodium iodosobenzoate.....	131
Loevenhart, A. S., and Grove, W. E. Studies on the pharmacological action of oxidizing substances.....	101
McGuigan, Hugh, and von Hess, C. L. Isocalycanthine and its quaternary base.....	441
McGuigan, Hugh, and Mostrom, H. T. Studies on the convulsive reflex pro- duced by strychnine. I. Habit.....	515
McGuigan, Hugh, and Mostrom, H. T. Studies on the convulsive reflex pro- duced by strychnine. II. As modified by epinephrine.....	521
Macht, David I. On the convulsant action of some dyes (Proceedings).....	471
Macht, David I. On the convulsant action of some sulphonated dyes.....	531
Macht, David I., and Abel, John T. Two crystalline pharmacological agents obtained from the tropical toad, <i>Bufo aguiar</i>	319
MacNider, William de B. A study of the action of various diuretics in uranium nephritis.....	423
MacNider, William de B. The action of diuretics in uranium nephritis (Proceedings).....	459
Mammalian heart, The action of caffeine on.....	609

Meek, W. J., and Eyster, J. A. E. The electrocardiogram in morphin poisoning in the dog (Proceedings).....	468
Meltzer, S. J., and Githens, T. S. On the duration of irritability of the motor nerves of the frog after death and the effect of strychnin upon it (Proceedings).....	463
Meltzer, S. J., and Joseph, D. R. On the convulsant action of acid fuchsin upon frogs deprived of their cardiac circulation.....	183
Meltzer, S. J., and Joseph, D. R. On a difference between the effects of intravenous and intra-aortic injections of curarin in frogs (Proceedings).....	465
Mendel, Lafayette B., Underhill, Frank P., and Renshaw, R. R. The action of salts of choline on arterial blood pressure.....	649
Mendel, Lafayette B., Underhill, Frank P., and Renshaw, R. R. The physiological action of salts of choline (Proceedings).....	457
Menten, M. L., and Crile, G. W. Comparison of anesthesia, in plants possessing a motor mechanism and in animals (Proceedings).....	467
Mostrom, H. T., and McGuigan, H. Studies on the convulsive reflex produced by strychnine. I. Habit.....	515
Mostrom, H. T. and McGuigan, H. Studies on the convulsive reflex produced by strychnine. II. As modified by epinephrine.....	521
Nephritides, Studies of renal function in experimental (Proceedings).....	460
New water soluble active constituent of squills.....	155
Nicotine, A note on the effect of, on adrenal secretion.....	379
Ott, Isaac, and Scott, John C. The action of glandular extracts upon the amount of epinephrin in the blood.....	625
Oxidative processes, The action of drugs on (Proceedings).....	472
Oxidizing substances, Studies on the pharmacological action of.....	101
Park, William H. The advantages of single over multiple doses of antitoxin and of intravenous over subcutaneous or intramuscular injections (Proceedings).....	474
Peritoneal and pleural absorption (Proceedings).....	460
Pharmacological action of oxidizing substances, Studies on the.....	101
Pharmacological action of vanadium.....	477
Phelps, I. K., and Salant, William. Demethylation of caffein and theobromin under pathological conditions (Proceedings).....	469
Phenolphthalein oxine, The physiological action of.....	161
Physiological action of cytisine, the active alkaloid of laburnum (cytissus-laburnum).....	205
Physiological action of phenolphthalein oxine.....	161
Physiological action of salts of choline (Proceedings).....	457
Physiological studies in anaphylaxis. IV. Reaction of the cat toward horse serum.....	299
Pilcher, J. D. Alcohol and caffein: a study of antagonism and synergism....	267
Pilcher, J. D. The action of caffein on the mammalian heart.....	609
Pilcher, J. D., and Sollmann, Torald. The actions of caffein on the mammalian circulation. I. The persistent effects of caffein on the circulation.....	19

Pilocarpine and atropine, The effects of, upon the amylolytic power and composition of the saliva.....	1
Protein poison, Upon the action of (Proceedings).....	461
Quantitative studies on the gastro-intestinal absorption of drugs. II. The absorption of sodium iodide.....	387
Rana pipiens, The seasonal and sex variations of, to digitalis (Proceedings)	458
Reaction of the cat toward horse serum.....	299
Reiger, J. B., and Salant, William. The elimination of caffein (Proceedings)	469
Reiger, J. B., and Salant, William. The toxicity of caffein (Proceedings).....	455
Renal function in experimental nephritides, Studies in (Proceedings).....	460
Renshaw, R. R., Mendel, Lafayette B., and Underhill, Frank P. The action of salts of choline on arterial blood pressure.....	649
Renshaw, R. R., Mendel, Lafayette B., and Underhill, Frank P. The physiological action of salts of choline (Proceedings).....	457
Respiration, The action of hydrocyanic acid on the, and the antagonistic action of sodium iodosobenzoate.....	131
Robertson, T. Brailsford, and Burnett, Theo. C. On the action of sodium citrate upon mammalia, with especial reference to acquired tolerance and to its action upon the cerebellum.....	635
Roth, George B. The action of quinine on leucocytes (Proceedings).....	470
Rowntree, L. G., and Dandy, W. E. Peritoneal and pleural absorption. (Proceedings).....	460
Rowntree, L. G., and Fitz, R. Studies of renal function in experimental nephritides (Proceedings).....	460
Rowntree, L. G., and Geraghty, J. T. Intramuscular versus subcutaneous absorption (Proceedings).....	460
Salant, William. The effect of caffein on the circulation (Proceedings).....	468
Salant, William, and Phelps, I. K. Demethylation of caffein and theobromin under pathological conditions (Proceedings).....	469
Salant, William, and Reiger, J. B. The elimination of caffein (Proceedings)...	469
Salant, William, and Reiger, J. B. The toxicity of caffein (Proceedings).....	455
Saliva, The effects of pilocarpine and atropine upon the amylolytic power and composition of the.....	1
Schultz, W. H. Physiological studies in anaphylaxis. IV. Reaction of the cat toward horse serum.....	299
Scott, John C., and Ott, Isaac. The action of glandular extracts upon the amount of epinephrin in the blood.....	625
Seasonal and sex variations of Rana pipiens to digitalis (Proceedings).....	458
Sodium citrate, On the action of, upon mammalia, with especial reference to acquired tolerance and to its action upon the cerebellum.....	635
Sodium cyanide, The influence of sodium iodoxybenzoate and, upon an allergic reaction of inflammatory character.....	223
Sodium iodide, The absorption of.....	387
Sodium iodosobenzoate, The action of hydrocyanic acid on the respiration and the antagonistic action of.....	131

Sodium iodoxybenzoate and sodium cyanide, The influence of, upon an allergic reaction of inflammatory character.....	223
Sollmann, Torald, and Hanzlik, P. J. Inhibition of iodide absorption by chloride (Proceedings).....	473
Sollmann, Torald, and Pilcher, J. D. The actions of caffein on the mammalian circulation. I. The persistent effects of caffein on the circulation.....	19
Squills, A new water soluble active constituent of.....	155
Strychnine, Studies on the convulsive reflex produced by.....	515, 521
Studies on the convulsive reflex produced by strychnine. I. Habit.....	515
II. As modified by epinephrine.....	521
Studies on the pharmacological action of oxidizing substances.....	101
Study of the action of various diuretics in uranium nephritis.....	423
Sulphonated dyes. On the convulsant action of some.....	531
Toxic action of amianthium muscaetoxicum (Proceedings).....	473
Toxicity of caffein (Proceedings).....	455
Underhill, Frank P., Renshaw, R. R., and Mendel, Lafayette B. The action of salts of choline on arterial blood pressure.....	649
Underhill, Frank P., Renshaw, R. R., and Mendel, Lafayette B. The physiological action of salts of choline (Proceedings).....	457
Uranium nephritis, The action of diuretics in (Proceedings).....	459
Uranium nephritis, A study of the action of various diuretics in.....	423
Vanadium, Pharmacological action of.....	477
Voegtlin, C. The action of drugs on oxidative processes (Proceedings).....	472
Wallace, George B. Chronic chloral poisoning (Proceedings).....	462



25. 17

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